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DATE FRUIT FIBER VARIABILITY IN COMPOSITION, TISSUE DISTRIBUTION, AND CONTRIBUTION TO HARDNESS OF DATE FRUITS (PHOENIX DACTYLIFERA L.)

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This dissertation is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Under the Supervision of Professor Afaf Kamal-Eldin

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Declaration of Original Work

I, Navomy George, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this dissertation entitled "*Date Fruit Fiber Variability in Composition, Tissue Distribution and Contribution to Hardness of Date Fruits (Phoenix Dactylifera* L.*)*", hereby, solemnly declare that this dissertation is my own original research work that has been done and prepared by me under the supervision of Professor Afaf Kamal-Eldin, in the College of Food and Agriculture at UAEU. This work has not previously been presented, published or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my dissertation have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this dissertation.

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Abstract

Date fruit (*Phoenix dactylifera* L.) is a major desert crop and is an integral part of the United Arab Emirates. This study is on the various aspects of dietary fiber of ten Emirati date varieties. The main objective of this dissertation is to analyze the content and composition of mature date fruits and to study their microstructure. The aspects of biomineralization with particular importance to silica phytoliths and lignification are also studied. Finally, the date fruit dietary composition and microstructure are related to the textural attributes of the fruit. Uppsala method of dietary fiber analysis (AOAC 994.13) is used to analyze the dietary fiber content and composition, along with Fourier transforms infrared spectroscopy. Light and scanning electron microscopic (SEM) methods are used to study the fruit microstructure, depositions of phytoliths (plant silica), and calcium oxalate crystals. Lignification and silicification pattern in various fruit tissues are observed by staining techniques and SEM. Finally, the date fruit fiber content and microstructure are correlated with date fruit texture. The total dietary fiber content in the date fruits analyzed is in the range of 5.2%–8.4%. Lignin is the major determinant of dietary fiber content in dates. Softer fruits contained lower levels of lignin, whereas increased lignin content was observed in harder fruit varieties. The light and SEM work revealed the heterogeneity and complexity in the silica phytoliths and the lignified structures in date fruits. Apart from their independent existence in the fruit tissue, a small proportion of lignin and silica seemed to co-exist as partners in the spiral coils of the tracheid phytoliths. Lignin, arabinoxylan, galactomannan, and pectin were found to correlate significantly with fruit hardness

Keywords: Date fruit, *Phoenix dactylifera*, dietary fiber components, lignin, texture, microstructure, silica, xylem vessels, sclereids, phytoliths.

Title and Abstract (in Arabic)

ألياف ثمار التمر: التباين في المكونات وتوزيع الأنسجة والمساهمة في صلابة ثمار التمر (*Phoenix dactylifera* L.)

الملخص

تعتبر ثمار التمر من المحاصيل الزراعية الرئيسية ولها دور مهم في ثقافة وتقاليد وأسلوب الحياة في دولة الإمارات العربية المتحدة. تهدف هذه الرسالة إلى تحليل محتوى وتكوين الألياف الغذائية في ثمار التمر الناضجة، ودراسة بنيتها المجهرية، وربط هذه المعلومات التركيبية بصلابة الثمار. تم اختيار عشرة أنواع من فاكهة التمر التي يتم تناولها بشكل شائع للدراسة. يتراوح إجمالي محتوى الألياف الغذائية في ثمار التمر بين 5.2-8.4% ويمثل اللجنين المكون الرئيسي للألياف الغذائية في التمر، وكلما زاد محتوى اللجنين زادت صلابة الثمرة. لقد لوحظ عن طريق الدراسات المجهرية ترسبات اللجنين مع السيليكا في أنسجة التمر وقد ارتبطت مكونات الألياف الغذائية المختلفة ارتباطات مختلفة مع صلابة الثمار.

مفاهيم البحث الرئيسية: فاكهة التمر، فينيكس داكتيليفيرا، الألياف الغذائية، اللجنين، السيليكا.

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Words fall short in expressing my gratitude to my beloved family. The compassion they shared and the love, support, and understanding they provided were

phenomenal. Above all, I bow my head in awesome reverence to the Almighty God who equipped me with all that I have to date.

Dedication

Dedicated to my family & friends

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AOAC	Association of Official Analytical Chemists
DF	Dietary Fiber
DPP	Days Post Pollination
DPX	Dibutylphthalate Polystyrene Xylene
DW	Dry Weight
EDS	Energy Dispersive X-ray Spectroscopy
FOS	Fructooligosaccharides
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GOS	Galactooligosaccharides
HMWDF	High Molecular Weight Dietary Fiber
IOM	Institute of Medicine
LMWDF	Low Molecular Weight Dietary Fiber
NFP	Non-Fibrous Polysaccharide
NSP	Non-Starch Polysaccharides
SEM	Scanning Electron Microscopy
TDF	Total Dietary Fiber

Chapter 1: Introduction

1.1 Dates and date dietary fiber

Dates are the primary agricultural produce of the arid and semi-arid regions because of their capacity to thrive in harsh desert conditions [1]. Date fruits are a source of energy food, rich in minerals like silica, iron, selenium, potassium, and magnesium [2-4]. They are a good source of carbohydrates, including dietary fiber, vitamins, and other antioxidants and are employed in systems of alternative medicine as an ingredient of tonics and ameliorant [5-8].

Dietary fiber in date fruits contributes to its functional and nutraceutical properties. Date fruits are a reasonable source of dietary fiber, especially the insoluble dietary fiber that provides its various health benefits [2, 3, 9, 10]. Along with various polyphenols, dates are considered to be a nutritious and healthy source of dietary fiber with functional attributes [7, 11, 12].

UAE is the 7th largest producer of dates in the world [13]. Hence, it is important to utilize the dates produced in the country to harvest the potential of date dietary fiber to the fullest. A thorough understanding of the content and composition of the dietary fiber in date varieties with different textures, which are commonly produced in the country, is critical in making effective use of the dates produced in the country, especially the wasted dates and the date waste from the various processing centers after extraction of the date syrup (dibs). This study aims at understanding the date dietary fiber and its components of Emirati dates.

1.2 Research objectives

The central aim of the research was to form a knowledge base about the dietary fiber content of 10 Emirati date fruits at full maturity, belonging to different texture ranging from soft to hard fruits. These are the commonly consumed table varieties in the country. They are also the varieties that are used in processing to produce date syrup (dibs) which is a major processed product from date fruits in the country. The specific research objectives were:

1) To determine the content of total dietary fiber in date fruits of varying hardness, analyze the component dietary fiber fractions, and know the primary determinant DF component of TDF. This was achieved by the Uppsala method, AOAC 994.13, together with FTIR spectroscopy.
2) Study the tissue distribution patterns and microstructure of date fruits using light microscopy and scanning electron microscopy.
3) To analyze the relationship between chemical composition, microstructure, and texture profile values of date fruits.

1.3 Thesis outline

The thesis comprises six main chapters. Chapter 1 provides a short introduction to the research, its specific objectives, and a general outline of the thesis.

Chapter 2 consists of a literature review providing the major works in general about dates, dietary fiber and the different DF components, and the nutritional significance of DF. The chapter also discusses the other analysis methods for dietary fibers and the biomineralization of date fruits. The chapter ends with a note on the use

of microscopy as a tool for dietary fiber studies concerning microstructure in plant tissue specimens.

Chapter 3 is on the quantitative dietary fiber analysis of date fruits by the Uppsala method and the qualitative dietary fiber studies by FTIR spectroscopy. The primary determinant for the TDF in date fruits was identified by correlating the TDF content with the component DF values. This chapter is based on an article, published in the NFS Journal.

Chapter 4 is about the microscopic studies of the silica phytoliths and the lignin deposition patterns of the date fruit. The date fruit microstructure was studied after trying different stains and fixation methods, by light microscopy. SEM studies were done on date fruits as well as on the extracted date fruit phytoliths. SEM/EDS imaged the plant digesta from the date fruits to obtain an elemental map for extracted date fruit phytoliths. This chapter is also based on an article published in the journal, Frontiers in Plant Science.

Chapter 5 examines the relationship between the chemical composition and microstructure to the various textural attributes of the date fruits obtained from texture profile analysis. The chapter is based on an article published in the Nature Research journal, Scientific Reports.

Finally, Chapter 6 presents the overall summary of the research works and the recommendations for future research.

Chapter 2: Literature Review

2.1 Dates

The date palm (*Phoenix dactylifera*) is a plant of the palm family – Arecaceae. The date palm has been cultivated since ancient times and is considered one of the oldest domesticated trees globally. Date fruits are supposed to be consumed by people during the Neanderthal age, as evident from the paleobotanic studies of fruit phytoliths [14]. Since the fruit shelf life could be extended by simple sun drying, it has been the staple food and major source of wealth in the Middle East and North Africa's irrigable deserts. In 2019, the worldwide production of date fruits exceeded 9.75 M tonnes. UAE is the 7th largest producer of dates with a production of 503421 tonnes of dates. The average yield of date fruits in the UAE is around 9480 kg/ha [15]. Date fruits are a rich source of carbohydrates including dietary fiber, vitamins, minerals, and polyphenols. They are grown in the arid and semi-arid regions of the world, especially in the desert oasis regions as a source of readily available energy, and are used in various systems of traditional medicine as a tonic and ameliorant [5-8].

Botanically a drupe, date fruit has a single tough seed, covered by a thin endocarp, a fleshy mesocarp, and an outer epicarp, which is the fruit's skin (Figure 1). Date fruits undergo several stages of development from pollination till it attains full maturity. They are hababouk stage, khimri/green stage, khalal/color stage, rutab/soft ripe stage, and tamr/full ripe stage (Figure 2). Date fruits differ extensively in their flesh weight, color upon maturity, texture, etc., between cultivars and smaller extend within cultivars [16].

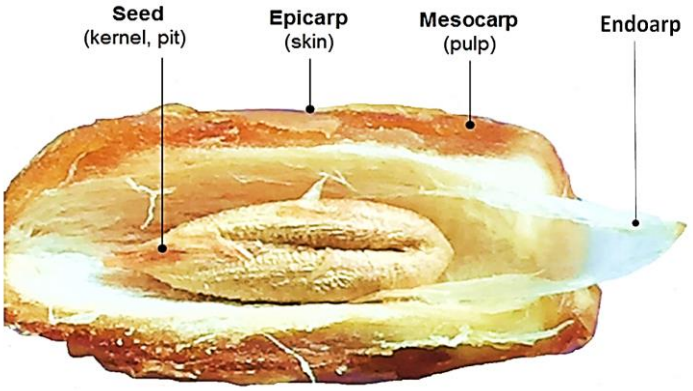

Figure 1: Date fruit at Tamar stage showing the epicarp, mesocarp, endocarp and seed. (Reproduced from [10]).

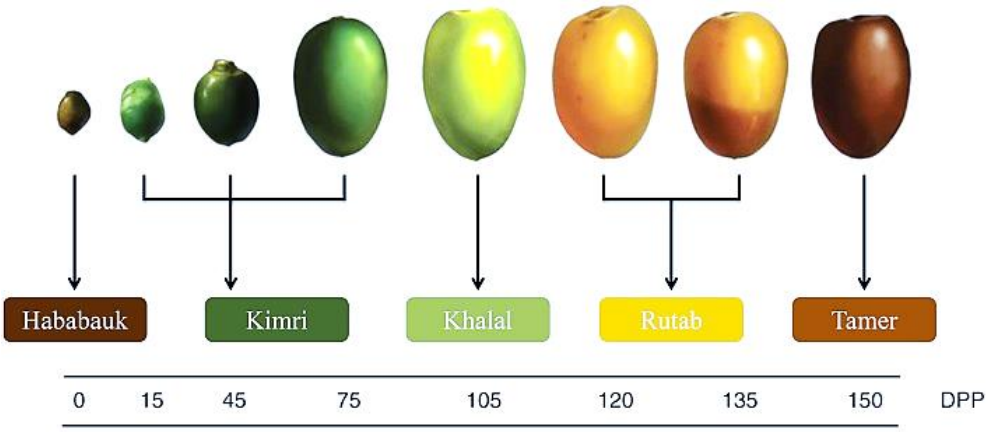

Figure 2: The five growth stages of a date fruit by days post pollination (DPP). (Reproduced from [17]).

Long E, as early as 1943, has described the anatomical characteristics of the date fruit. Anatomically, the fruit shows epidermis, hypodermal layer, a row of stone cells, followed by outer mesocarp and a section consisting of tannin-filled cells, followed by inner mesocarp and endocarp before the ovule or seed. As the fruit attains maturity (tamr stage), the mesocarp parenchyma walls become thinner and weaker since they are hydrolyzed, and some cells even undergo complete wall dissolution.

Softening starts at the fruit apex and proceeds to the fruit base and anatomically, from the epicarp to the endocarp [18-20]. Thus, during the tamr stage, the fruit loses moisture, and the concentration of sugars increases, which prevents the fermentation of the fruit, just as in the case of other dried fruits like raisins or dried prunes. The water content of the fruit will decrease to 40-60% as the ripening initiates, from around 80% in the young fruit. This loss of moisture accelerates rapidly as the fruit attains full maturity and the total dry matter increases from 20% in the early Kimri stage to around 72-88% at tamr (full maturity) [20]. Thus, the final sweetness and texture of the fruit depend on the fruit variety, stage of maturity, and ripeness at which the dates are harvested [6, 21]. Date fruits are a source of high-energy food, rich in sugars, mainly fructose and glucose. They are low in protein and fat but are packed with minerals like silica, iron, selenium, copper, magnesium, and potassium [2, 3, 7].

Date fruits are a good source of DF with around 4.7-12.7% of fruit, of which insoluble fiber is 84–94% and soluble dietary fiber is around 6-16% [2, 3, 10, 21]. The size, shape, weight, texture, etc., of the date fruits vary based on their variety and growing conditions [5, 21]. Dietary fibers in dates constitute the functional properties in date fruits and are important in providing health benefits along with the presence of antioxidants [2, 9]. Cellulose, some hemicelluloses, and lignin are the insoluble DF fractions in date fruits. Pectin, various fructooligosaccharides, inulin, galactomannan, β-glucan, etc. are the significant soluble DF fractions [11, 21-23]. Date fruits are also rich in phenolic and carotenoid content. Hence, with their inherent antioxidant properties, dates are considered a nutritious and healthy source of DF with functional attributes [7, 11, 12]. The date fruit is also a rich source of various phytochemicals like phenolics, sterols; vitamins and provitamins; and flavonoids. The amount of these

varies based on the type of the fruit, stage of fruit harvest, location, and soil and agronomic conditions [7, 8, 24, 25]. The nutritional and organoleptic properties of the fruits are determined by the content and ratio of various components like moisture, dietary fiber content, and phenolic compounds [26].

Date fruits are generally classified as soft, semi-dry, and dry varieties based on the perceived fruit hardness of the edible part and their moisture contents at the fresh Tamr stage. Soft varieties have around 30% moisture content, semi-dry varieties are with moisture content in the range of 20–30% and, dry varieties have less than 20% moisture [10]. Varieties like Barhi and Lulu are soft varieties, while Khalas, Shishi, and Sagei are semi-dry varieties. Neghal is a popular dry date variety in the Middle East region. The content of DF in date fruits is comparable to other popular dried fruits. Table 1 shows the DF quantities in some of the commonly consumed dried fruits [27]. The DF content in dates, including cellulose, hemicellulose, pectin, and lignin will decrease as the fruit progresses from the rutab to the tamr stage [3]. As discussed earlier, all date varieties show decreased moisture content and increase in sugar contents as the fruit matures. This enhances the shelf life of the fruit and thus dates fruits with the combined utility of nutrients being provided along with excellent shelf life, make it a remarkable dietary component [5, 20].

Table 1: Dietary fiber in other dried fruits

Fruit	**Dietary fiber (g/100g)**
Dried blue berries	7.5
Raisins	6.8
Apricots	7.3
Pears	6.4
Cranberries	5.3
Figs	9.8
Plums (prunes)	7.1
Peaches	8.2

2.2 Fruit texture and its significance in dates

Food textural properties are defined as “the group of physical characteristics that arise from the structural elements of the food that are sensed principally by the feelings of touch, are related to deformation, disintegration, and flow of food under force, and are measured objectively by functions of mass, time, and distance” [28]. Texture and the consumer acceptance of fleshy fruit are determined by the mechanical and chemical properties of the fruit cell walls. During ripening chemical and hence structural changes take place in fruits which results in softening of the texture of the fruit [29]. In fruits like pears, the nanostructure of dietary fiber molecules of the cell wall like pectin, hemicellulose, and cellulose affects the textural properties of firmness [30]. The texture is an essential factor in the determination of various processing parameters and conditions of a fruit. Thus, mechanical and chemical properties of

various cell wall components affect the texture of the fruit at microscopic and macroscopic levels.

Date fruits at full maturity (Tamr stage) are primarily composed of sugars (60–80%), with the remaining of the fresh weight being moisture (10–30%), dietary fiber (5–12%), phenolic compounds (up to 4%), and other minor constituents [10]. Two main factors are considered important when classifying dates in terms of perceived texture. They are, type of sugar at the time of harvest, i.e., sucrose ratio to reducing sugars and the moisture content. Dates with more than 30% moisture at the time of harvest are soft in texture while dates with 20-30% moisture are semi-dry in nature, and dry dates contain only less than 20% moisture at harvest [31, 32]. However, sugar content in dates did not contribute to the textural properties of the fruit in statistical clustering studies done on 20 date varieties of the UAE, although texture was not found to be the main determinant of classification of the date fruits [16].

Most of the date varieties grown in the Middle Eastern region are the soft and semi-dry varieties which are closely related except for minor genotypic and few phenotypic factors like agronomic practices and environmental conditions [31, 33]. Amount and type of insoluble, soluble dietary fiber and phenolic compounds can also attribute to the textural difference in dates [34, 35]. Cell wall structure, especially the shape and size of the cells of skin and underlying pericarp tissue layers, also may affect the texture of date fruits [36, 37].

2.3 Dietary fiber and its nutritional significance

Dietary fiber, once considered as an unwanted and insignificant component in the human diet, has gained significant nutritional importance because of its

nutraceutical properties and significant influence in various body functions like cardiovascular health, insulin regulation, body weight, and gastrointestinal health benefits. Research on dietary fiber started some 60-70 years after Hipsley first used the term, to describe the plant cell walls in the diet [38]. However, a meaningful definition to the term was given later, when the concept of the physiology of the ingested fiber component was added and related to health benefits due to the effect of indigestible carbohydrates on lipid metabolism was observed. Thus, the term DF was redefined, wherein lignin was included along with indigestible polysaccharides [39]. Resistance to the action of digestive enzymes in the small intestine is still the at core of any definition of DF.

Nevertheless, with the advancement of newer scientific technologies and analytical methods, more and more components are added to the classification of dietary fiber. Hence, a straight and short definition for the term is almost impossible, and most definitions are based on the various applications of the respective fields. DF cannot be defined as a single chemical entity or a group of related compounds. Since all different types of carbohydrates are separated at different levels of complexity, and the definitions and analysis of DF are related; the conceptual definitions are to be in accordance with the various analytical methods employed [40-43]. However, the basic concepts on which dietary fiber is defined from a human food perspective is that they should be edible components from plant sources - polymers - including oligosaccharides, polysaccharides and/or polyphenolic compounds; resistant to hydrolysis by human gut enzymes, thus resistant to absorption in the small intestine, but can undergo hydrolysis and partial or total fermentation by the microbes present in the large intestine [44].

In 2009, the Codex Alimentarius commission put forward a detailed definition of dietary fiber to bring together the various definitions of DF, describing what all compounds can be considered as dietary fiber from the nutritional point of view. In short, the definition is applied to carbohydrate polymers that have ten or more monomeric units and are not hydrolyzed by the endogenous enzymes in the small intestine of humans. These belong to various categories like edible carbohydrate polymers naturally occurring in the food; carbohydrate polymers obtained from food substances by any mode of physical, enzymatic, or chemical methods of extraction; and synthetic carbohydrate polymers. Such an extracted polymer should have an acceptable physiological health benefit that can be proven scientifically. However, the freedom of decision on whether to include the oligomers with 3-9 monomers in the DF category is given to respective countries [45, 46]. DeVries provided a list of events from the start of usage of the term till various developments in the field have taken place [40]. Stephen and other authors provided an extensive review on dietary fibers, which encompasses the various present-day definitions of dietary fiber from the time the term evolved. It also covers the aspects of DF analysis methods, health effects, and the impact of different DF components on health [47].

Fruits are rich in dietary fiber, antioxidants, and phytochemicals that are proved to have various health benefits. Systematic reviews and meta-analysis have proved that increased fruits and vegetable consumption results in an improved general diet profile because of enhanced intake of various micronutrients, vitamins, dietary fiber, carbohydrates, and minerals [48] and is inversely associated with cardiovascular disease risks [49]. Many modern-day diseases like those affecting the proper function of the heart and gut are shown to be related to the amount of time taken for the gut

contents to pass through the alimentary canal and on the consistency of the same. This nature of the gut contents and the time taken for its movement along the digestive tract is directly proportional to the DF content in the ingested food. DF in food also affects the total calorie intake, nature and population strength of colonic microbes, blood cholesterol, and bile metabolism; all of which have a direct impact on general health and well-being [50, 51].

Although several epidemiological studies related to diet patterns, the composition of food categories, and nutrients have pointed out a notable association with dietary fiber intake and general health and well-being, DF is often considered as a single entity. However, DF is an umbrella term consisting of different compounds which are different in their biological and chemical properties, ranging from non-digestible oligosaccharides to cellulosic materials of the plant cell wall [52]. DF from various sources has shown to have a variety of health benefits like balancing blood glucose levels, improving bowel movements, reduction in blood cholesterol levels, and lessening the troubles of gastro-intestinal tract disorders like ulcerative colitis, Crohn's disease, and other inflammatory bowel diseases [51, 53]. Higher intake of fruits and vegetables is proved to considerably reduce the risk of major health-related mortality causes like cardiovascular diseases and cancer [54]. DF intake reduces chances of cardiovascular diseases by various mechanisms which involve the reduction of insulin resistance, reducing inflammatory markers, cholesterol reduction by binding the same with bile and helps in expelling it from the body, thus preventing reabsorption; improved gut microflora growth and balance which help in better weight control [55, 56].

Studies have proved that a ten-gram increase in total DF intake can significantly reduce the mortality risk due to various heart diseases, cancers, and all-cause mortality, including various infectious diseases. The beneficial effects on mortality are shown to improve progressively as a function of fiber consumption through improved serum lipid concentration, immune functions, glucose absorption, insulin sensitivity, etc. [57]. DF in the diet improves health by increasing the fecal bulk, improving fecal viscosity, and decreasing contact time for inflammatory agents and carcinogens in the intestinal tract. Dietary fiber enhances the production of short-chain fatty acids, the binding between bile acids and the supply of antioxidants to the body [58]. Thus, DF is vital in providing gastrointestinal wellbeing by lowering constipation, mitigating various digestive tract health issues like irritable bowel syndrome, inflammatory bowel disease, and diverticular disease.

Recent studies have proved the influence of gut microflora, particularly its diversity on physical and mental wellbeing. Evidence from various studies has associated beneficial gut microbe strength to gastrointestinal diseases and also to extra gastrointestinal diseases. Psychological issues like anxiety, depression, etc. can be linked to imbalance or reduction in gut microbes [59]. Long-term benefits of adequate DF consumption from whole fruits can bring about various health benefits including bodyweight management, improved cardiovascular health, reducing the chance of type two diabetes and other metabolomics syndromes, various cancers, and other inflammatory diseases. Consumption of whole fruit fibers is proved to reduce the aging process and enhance psychological wellbeing by enhancing the diversity and population of the gut microflora [60]. The Institute of Medicine’s recommendation is a daily intake of 25 grams and 38 grams of DF for women and men of 19-50 years of age [61].

2.4 Dietary fiber components

The material that enters the large intestine without undergoing digestion and absorption in the small intestine is mainly plant cell wall material, or those materials that are entrapped in the cell wall, except for resistant starches and other novel derived fiber forms. Thus, the major source of the dietary fiber in human diet is the fraction that is derived from the plant cell wall and the components include non-starch polymers like cellulose, hemicellulose, pectin, gums, mucilages, etc., non-digestible oligosaccharides like inulin, fructans, etc., and non-carbohydrate polymers like lignin, and associated minor substances, such as waxes, cutin, suberin, saponins, polyphenols, phytates, and phytosterols. The inclusion of fractions from associated compounds is determined based on the analytical methods employed [40, 41, 47, 62, 63]. Cell walls of plants make up a major part of DF obtained from any plant source, be it fruits or vegetables.

The plant cell wall, from which the significant fraction of insoluble DF is derived in any plant-based diet, is composed of various macromolecules structured and arranged marvelously by nature. They play an appreciative role in determining the various quality characteristics of such foods, especially in food texture, digestibility, and bioavailability [64]. The cell walls perform various functions like providing structure and strength to the cell, protect the cells from pathogen invasion, and many other biochemical and physiological functions [65]. Irrespective of the part or organ to which it belongs, plant cells are of three basic types – parenchyma, collenchyma, and sclerenchyma cells. Tissues may be simple, with only one of the above-mentioned types of cells or complex, as in the case of xylem [66].

The outer skin layer or epidermis of aerial organs of all vascular plants is covered by a waxy cuticle. Thus, the epidermal skin layers of all fruits usually contain cell walls that are lignified and are protected by a layer of cuticle and wax layer. This layer prevents excess moisture loss from the fruit along with its protective roles from pests, diseases, and various weather conditions outside. The polysaccharides embedded in the cuticular matrix are supposed to have a role in keeping the structural integrity of the cuticle and, in turn, maintaining the structure of the fruit. The outer perimeter of most of the plant organs, which may get exposed to mechanical stress, is lined with sclerenchyma cells below the cuticle layer. The outer skin layers of the fruit may also consist of sclereid cells where the cell wall is thickened with lignin deposits upon maturity. This layer might play an essential role in maintaining the shape of the fruit, especially in soft and sugar-rich fruits like dates. The fruit mesocarp usually consists of parenchymatous cells in which a large volume is occupied by the cell vacuole. The vacuole is filled with sugars, starch, gums, resins, plant sap, or polyphenolic compounds like condensed tannins or anthocyanin based on the place of occurrence, growth stage, and fruit type. Thus, parenchymatous cells are the primary storage tissue in fruits. The cell walls of these cells are made up of cellulose and are lignified in variable degrees. Another tissue type is the collenchyma, where the primary cell walls are thickened to variable extends. The cell walls of collenchyma are made up of cellulose and pectin. However, contrary to the parenchymatous or sclerenchyma cells, the collenchyma cells are thickened without lignin.

Figure 3 shows the major plant tissue types discussed. Whether lignified or not, the majority of these cell walls are difficult to digest by the human digestive system and thus forms the DF, which is appreciated for its functional properties in the human diet [67-69].

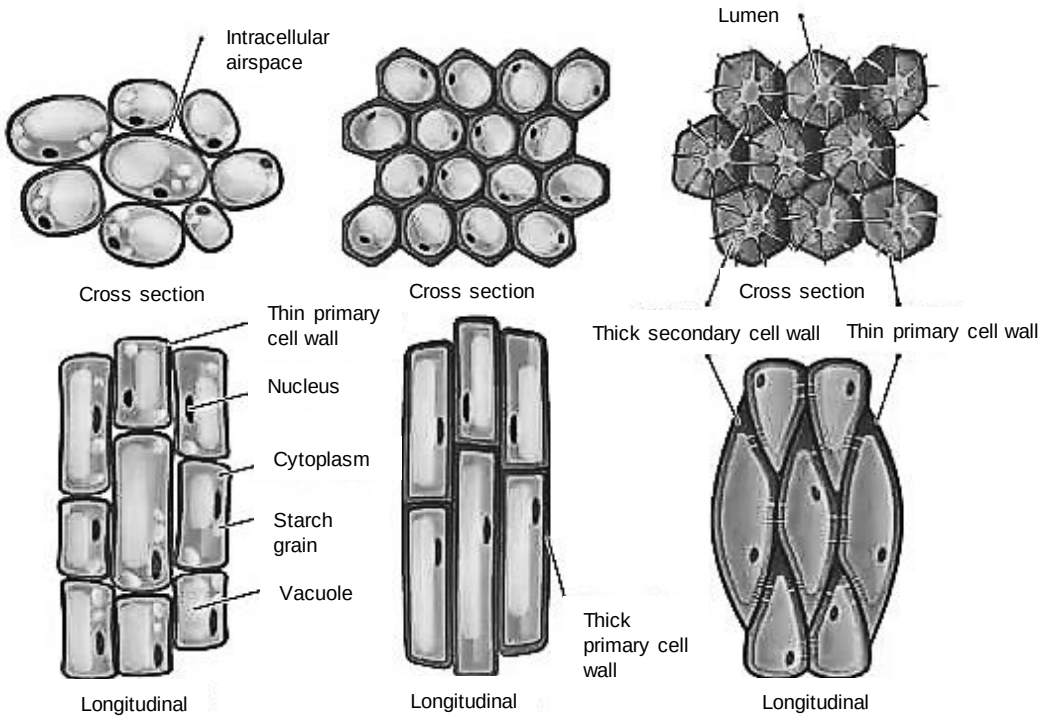

Figure 3: Plant tissue types (reproduced from [70]).

Plant cell walls are complex structures, where a composite structure is upheld by various components by various intermolecular bonds. Although the chemical structure of the individual dietary fiber component is mostly known, the understanding of the plant cell wall structure is not yet complete [67]. The basic building blocks of plant cell walls are cellulose, hemicellulose, pectin, and lignin. Thus, plant cell wall is a heterogeneous and dynamic structure in which cellulose microfibrils are interwoven into a complex polymeric matrix. Figure 4 is a schematic representation of how the various dietary fiber component polymers are chemically associated in a plant cell wall. The primary cell wall layer composed of cellulose fibers in a hemicellulose and pectin matrix is first secreted where hemicellulose binds to the surface of the cellulose microfibrils and pectin cross-links the hemicellulose molecules of adjacent

microfibrils. Glycoproteins also take part in the cross-linking process. Neighboring cells are attached together by pectin containing middle lamella. Cellulose fibers in a matrix of lignin and hemicellulose are deposited in some tissues as secondary layers, which may differ in thickness. Lignification increases the strength and stiffness of the cell walls and in some mature cells, the lignified regions may continue to provide mechanical support to the plant organelles even after the cells die away [69, 71].

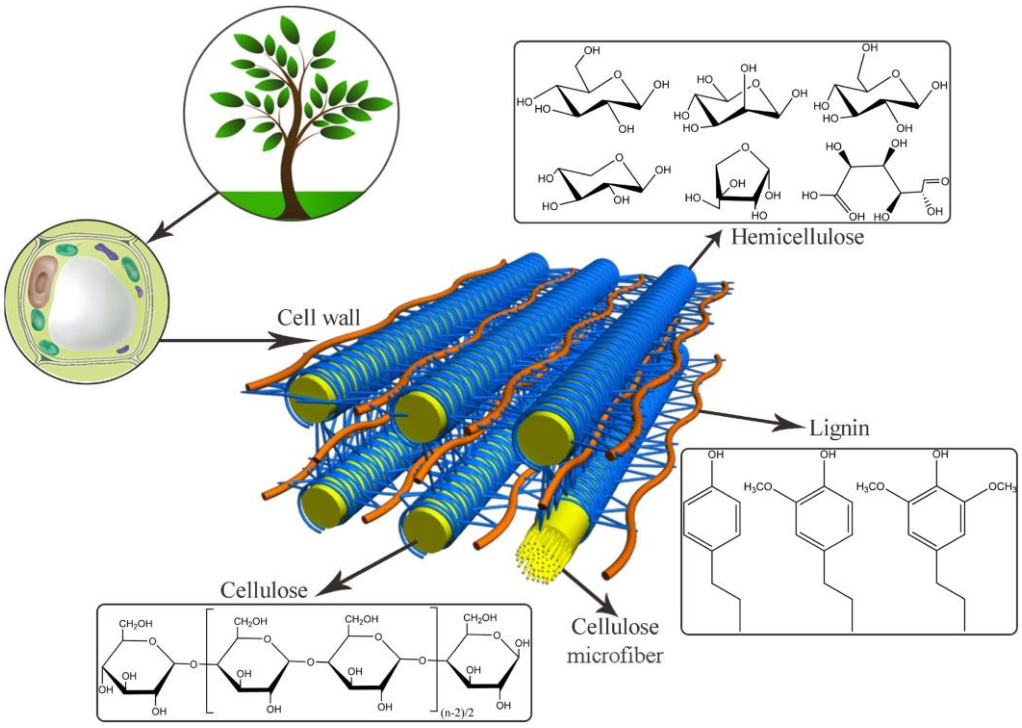

Figure 4: Composition and chemical structure of plant cell wall components. (Reproduced from [72]).

Cellulose, with its remarkably high mechanical properties, forms the main structural element. Cellulose is the most abundant natural polysaccharide, which forms about 20 -50% of dietary fiber in plant-derived foods [63, 73]. This linear and unbranched polymer of glucose is not digested by human enzymes but is fermented partially by the gut microflora to produce beneficial short-chain fatty acid moieties in the human colon. Cellulose can bind water, thus increasing the fecal volume, which

helps in promoting regular bowel movements [63, 74]. The cell wall microstructure variations and the cellular structure give rise to an extensive range of mechanical properties to plant cell walls. In the plant cell walls, cellulose molecules merge to form microfibrils, and they group into macro fibrils wherein the space between the microfibrils are occupied by a matrix of hemicellulose and either pectin or lignin, along with structural proteins and thus reinforces the entire matrix complex [67].

Hemicelluloses are short-chain polymers than cellulose but are heterogeneous, containing various monomers other than glucose. Hemicelluloses are amorphous polysaccharides and include xyloglucans, xylans, mannans, glucomannans, and galactoglucomannans [67, 74]. Depending upon the nature of the polymer, hemicelluloses may be soluble or insoluble. They form about 30% of the dietary fiber of plant-derived foods and are nutritionally crucial because of their cholesterol-binding property, which prevents its reabsorption. They are acted upon by the gut microbes to produce beneficial short-chain fatty acids and help regulate bowel movements due to improved hydration of stools [63].

Pectins are branched plant cell polysaccharides rich in galacturonic acid units along with rhamnose. Pectin is formed in the plant cell walls, especially in the outer skin or rind of fruits and vegetables, and is a significant component in the middle lamella, where they act as intercellular cementing aids of plant cells. Neutral sugars like arabinose, galactose, xylose, rhamnose, and glucose sometimes attach to pectin as side chains by covalent bonding [74]. Pectins are essential for human health as they can bind cholesterol by forming a gel along with bile acids, thus aids in excreting the excess cholesterol and prevent its reabsorption to the blood [63].

Non-digestible oligosaccharides are fructooligosaccharides (FOS) and galactooligosaccharides (GOS), and inulin. Beneficial bacteria digest them in the

human gut to produce short-chain fatty acids, which are absorbed by the body. Thus, they increase the number of colon-friendly bacteria and prevent the growth of colon degrading pathogenic microorganisms. They are considered dietary fiber because of their ability to deliver the same physiological functions as larger polysaccharides [75]. Fructans are soluble DF, which are oligomers of fructose, mostly linear, with β-1,2 linkage, with or without a terminal glucose moiety. They occur naturally in edible and non-edible plants. They can also be synthesized from saccharose. These DF components have continually proved the capacity to selectively encourage the growth of certain beneficial gut bacteria like Bifidobacteria, which has demonstrated positive effects in controlling pathogenic gut microbes and obesity [76]. Similarly, some carbohydrates travel through the small intestine without being digested and undergo fermentation in the large intestine. Resistant starches, fructooligosaccharides, galactooligosaccharides, modified celluloses, and synthesized carbohydrate polymers, such as polydextrose, are such carbohydrates. They also are categorized as dietary fibers with marked physiological effects [77].

Lignin is a complex phenolic compound and is amorphous in nature. Being, an integral component of the plant cell wall, it is the most abundant compound in the biosphere - second only to cellulose. Lignin was once considered as an anti-nutritional component and was thought to have only structural role in plants [78]. In the plant cell wall, lignin not only provides rigidity, strength, and form, but also controls the permeability of the cell wall, thus protecting the cell from moisture loss and preventing the easy access of pests and disease infestation. These characteristic properties are achieved by fixing the various cell wall polymers in place, thus excluding water content, and making the cell wall more rigid and recalcitrant. Coniferyl, p-coumaryl, and sinapyl alcohols polymerize to from lignin., and it will covalently link to

polysaccharides - directly by sugar residues and indirectly through ferulic acid esterified to polysaccharides [67, 79-81]. Lignin prevents the intestinal microbial digestion of plant cell wall fractions in the food by preventing their enzymes from reaching the target polysaccharides. Lignin is found to have free radical scavenging properties [82]. Table 2 gives a quick overview about the major dietary fiber components from plant sources and their constituent chemical groups.

Table 2: Major plant dietary fiber constituents and their chemical components

Fiber component	**Description**	**Main chain**	**Branches**
Cellulose	Linear polysaccharide of glucose units.	β-(1,4) glucose	
Hemicellulose	Hetero polysaccharide containing sugars other than glucose. a. Xylans b. Arabinoxylans c. Mannans d. Glucomanns e. Galactoglucomannans f. Galactomannans g. Xyloglucans	 β-D-(1,4) xylose -do- β-D-(1,4) mannose β-D-(1,4) mannose, β-D-(1,4) glucose -do- β-(1,4) mannose β-D-(1,4) glucose	 Arabinose Galactose α-D-galactose α-D-xylose
Non digestible oligosaccharides	a. Fructans b. Galactans c. Inulin	fructose with a glucose unit at any one end, joined by β-(2-1) glycosidic bond	inulobiose, levanobiose, and sucrose
Lignin	Non-carbohydrate phenolic polymer component associated with plant walls.	Polyphenols: *p*-coumaryl alcohol, Syringyl alcohol, Guaiacyl alcohol	

Table 2: Major plant dietary fiber constituents and their chemical components (Continued)

Fiber component	Description	Main chain	Branches
Pectins	Polysaccharide common to all cell walls, especially in middle lamella.		
	a. Homogalacturonan	α-(1,4)-D-galacturonic acid (with a few carboxyl groups that are methyl esterified)	
	b. Rhamnogalacturonan-I	(1,4) galacturonic acid, (1,2) rhamnose and 1-, 2-, 4- rhamnose	Galactose, xylose, arabinose, rhamnose and galacturonic acid
	c. Rhamnogalacturonan-II	α-(1-4) galacturonic acid	Sugars like - apiose, aceric acid, fucose
	d. Xylogalacturonan	α-(1-4) galacturonic acid	xylose
Beta-glucans	Branched polymer made of glucose	β-(1,4) glucose, β-(1,3) glucose	Glucose
Waxes, cutin and suberin	Micro components of the plant cell structure, especially in the epidermal layers.		

2.5 Dietary fiber analysis

Dietary fiber analysis started with the assessment of the digestibility of animal feeds and forage crops. These were crude measurements, and there were many uncertainties because of the single value of carbohydrate derived. Dietary fiber in human nutrition is to be measured and analyzed, keeping in mind its various chemical compositions and nutritional impacts, thus as a sum of various chemically identified dietary fiber components. The classification of dietary carbohydrates base on its

digestibility in the human gut is given in Figure 5. The main challenge in the analysis of dietary fiber from a human nutritional point of view is the implementation of chemical, physical and enzymatic approaches together in an analysis method to achieve the proper measurement of each dietary fiber fraction. This is why it is always superior to employ rational methods that involve measuring the specific compound of interest rather than using methodology-defined analysis (empirical methods) or proximate analysis. An essential dietary fiber analysis method involves proper sample preparation, which involves preparing a homogeneous sample maximum close to how it is consumed originally; its complete extraction and dispersion to effect proper hydrolysis and detection of component fractions by chromatographic or colorimetric assays [83]. However, because of the diversity in the chemical nature of the component dietary fiber fractions present, a single analytical method that gives an accurate and comprehensive result for dietary fiber analysis on nutritional and chemical aspects of the consumed food is still not available. Lignin is such an example of a dietary fiber component. Because the determination of the actual structure of an isolated molecule of lignin plant cell wall is not yet completely done and hence there is no standard lignin structure available for reference, and also because of its intricate linkage to other cell wall polymers, measurement, and calculation of lignin content is empirical and is very much dependent on the methodology employed [84]. Thus, the principle applied in dietary fiber analysis is a combination of gravimetric, enzymatic, and chemical techniques.

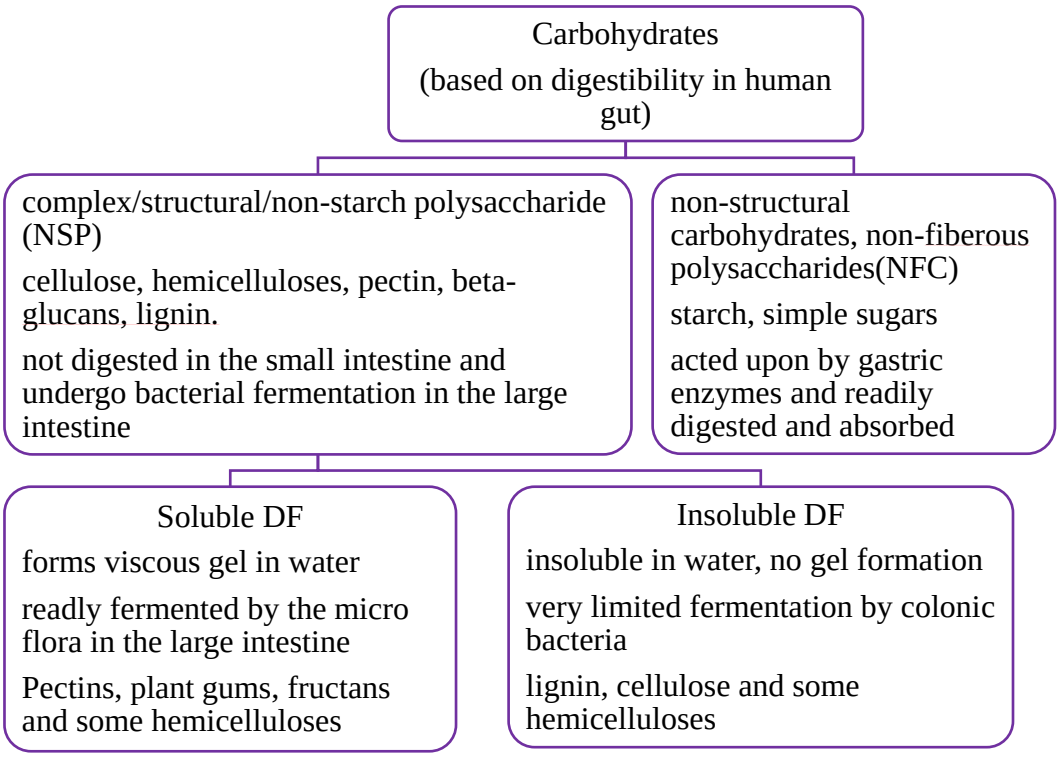

Figure 5: Classification of dietary carbohydrates based on digestibility in the human gut

Analysis of dietary fiber is a cumbersome and lengthy process. The molecular and physical characteristics of dietary fiber can differ extensively, depending on the specific source, method of isolation, food processing, and matrix [85]. Hence, the methods employed for the dietary fiber analysis largely depend on the fractions to be analyzed and the type of food matrix. In the case of cereals, the high starch content can interfere with the analysis, and in the case of fruits, the high sugar content delivers difficulty in the proper estimation and quantification of the amount of different dietary fiber fractions. Some dietary fiber fractions like soluble fibers can be removed while washing off the sugars. So, a careful method selection and modification of methods based on the food matrix and the available literature of dietary fibers, especially in high sugar-containing fruits.

Developing methods on dietary fiber analysis has two underlying aspects. The understanding of dietary fiber physiology has been in parallel to and dependent upon the developments in the progress of dietary fiber analysis methods. Since dietary fiber is a fraction of total dietary carbohydrates, any methods of analysis of DF should be consistent with the corresponding methods of analysis for other carbohydrates. The actual definition of dietary fibers was amended over the years, right from 1953 when Hipsley coined the term. Thus, the various fractions coming under the term were improvised over the years. This added to the improvements of the existing DF analysis methods and led to the development of new methods. Below is a table (Table 3) comparing some of the commonly employed dietary fiber analyses based on the various dietary fiber components measured in the particular method.

Table 3: Measured dietary fiber component in major DF analyses

Measured component	Analysis based on:
Total dietary fiber	AOAC 985.29
Total DF, soluble & insoluble DF	AOAC 991.43
Total DF, high and low molecular weight DF	AOAC 2009.01
Total, soluble & insoluble DF, HMWDF, LMWDF	AOAC 2011.25
β-glucan (cereals)	AOAC 995.16
Galactooligosaccharides	AOAC 2001.02
Inulin/Fructooligosaccharides	AOAC 997.07, AOAC 999.03
HMWDF, Uronic acid residues & Klason lignin	AOAC 994.13

Uppsala method (AOAC 994.13) is an approved method for the determination of TDF content in foods based on the various dietary fiber components measured, viz., total high molecular weight dietary fiber moieties as neutral sugars. Uronic acid residues, and Klason lignin are determined by spectroscopic and gravimetric method

respectively, after the starch and simple sugars are removed from the food matrix. The three values are added together to obtain the TDF in the given sample [86]. This method is called a defining method, which means a method used to determine the value of a particular component can only be achieved by that method. Thus, the aforesaid method will be the only way to obtain the acceptable value of the particular item [87].

2.6 Biomineralization of calcium and silicon phytoliths

Biomineralization in different organs and tissues deals with mechanisms and reasons by which plants assemble and use different minerals like silicon and calcium [88]. As in animals, biominerals are proved to provide plants with toughness, mechanical strength and flexibility, and may have functional roles as well, including protection against various biotic and abiotic stresses. Calcium oxalate crystals and amorphous silica are the commonly reported biominerals in plants. Depositions of minerals occur in most plant organs. The processes are assumed to have physiological functions and ecological significance [89]. Biomineralization can be extracellular, such as the deposition of silica in the cell walls or surrounding areas, or intracellular, such as the deposition of calcium oxalate in cell vacuoles. Biomineralization involves complex nucleation and growth biochemical processes that are controlled by genetic as well as physicochemical conditions. It is assumed that an organic matrix provides an initial contact point for the seeds of biomineral assemblies. While these processes are not yet fully understood, the resultant biominerals present defined crystalline structures in different plant species [90].

Calcium is an essential element in plants that is involved in several cellular processes. Excess calcium is deposited in different forms in plants, including calcium oxalate crystals, or raphides, which accumulate in the vacuoles of idioblasts cells of

the parenchyma [91, 92]. The production of calcium oxalate crystals increases with increasing calcium concentration in the growth medium, and they act as calcium reserves. Excessive free calcium in the cytoplasm is toxic to plants and interferes with several metabolic processes. Thus, the formation of calcium oxalate crystals will act as a calcium sink in the plant, modulating the availability of the element [88, 89].

In many plant genera of the plant kingdom, the occurrence of dense silica particles within cells and in intracellular spaces is reported. They occur in different shapes and sizes in well-defined tissues of leaves and stems of various monocotyledonous plants [93]. Spherical silica bodies and bundles of calcium oxalate needles – raphides in special sack-like cells were reported as cell inclusions in vegetative organs of young palms present abundantly in leaves and stem [94]. The occurrence of spherical silica bodies and calcium oxalate needles are reported in adult palms [95]. Both studies reported the presence of a conical or hat-shaped silica body with a flat base in the palms studied. Thus, it is assumed that the occurrence of these mineral structures starts at a very young age of the palm and proceeds as the palm matures.

Silicon was considered beneficial to a wide variety of plants, although not considered as an essential nutrient. The element as such, or as silica gel or silica bodies in plants, did not prove to have any role in plant metabolism. Thus, the role of Si in plants was once considered as purely mechanical and not physiological and as those which are expressed only under circumstances where plants experienced some kind of stresses [96]. Plants absorb silicon as silicic acid $Si(OH)_4$, dissolved in water. The silica taken up by the plant is deposited as amorphous form, either in a hydrated colloidal form or as a dehydrated condensed form of polysilicic acid [97]. The role of

Si, absorbed as monosilicic acid, is considered to be a priming factor for plants, wherein the Si acts as a tonic which keeps the plant ready to cope up with various stress presented by the environment in which the plant is grown; and when the stresses are absent, it takes a latent role [98]. Later, as research advanced, scientists proposed a newly hypothesized model for the role of silica in plants, considering its various beneficial effects and influences on plants. The apoplastic obstruction hypothesis positions Si as an extracellular prophylactic agent against various abiotic and biotic stresses with significant cascading effects on the form and function of plants [99].

The monosilicic acid absorbed by the plants condenses due to transpiration, and the supersaturated solution thus formed will start to polymerize and solidifies. The dehydrated, condensed forms of absorbed silicic acid ($SiO_2.H_2O$) in the intercellular and intracellular spaces of plant tissue are known as phytoliths, earlier called plant opals [100]. Phytoliths are produced in plants and serve various functions, and they are incredibly resistant to decomposition. Phytoliths on dental calculus from excavated fossil remains of human skulls provide evidence of what people ate during the early years of history and their subsistence on plants, including date fruits [14].

Silicon is found to have a profound role in the deposition of lignin in plant cell walls. The presence of a controlled association between the accumulation of silica and lignin deposition in plants has been suggested [101]. The gene-regulating phytolith development in plants also regulates the lignin production [102]. A significant association exists between lignin and silica. Silicon is considered to induce the formation and accumulation of lignin. It also has a great affinity for organic polyhydroxyl compounds involved in the biosynthetic pathways of lignin. Previous histological analyses show intense lignin accumulation in plants treated with silicon in

regions of the vascular bundle and the sclerenchyma layer beneath the epidermis. Silicon treatment has been shown to increase the lignin content and formation of silicified microstructures [103]. The availability and presence of silicon are supposed to have resulted in an added preference for organic compounds participating in lignin biosynthesis, thus resulting in increased buildup of lignin in plants [103]. Date palm is a silicon accumulator, with tissues of roots, stem, and leaves having up to 1% of silicon content as phytoliths, mainly in the sclerenchyma of vascular bundles. However, the association of silica with lignification was ruled out [104].

2.7 Microscopy as a tool for dietary fiber studies

Plant cell walls being the major component of dietary fiber in plant-based foods, microscopic studies can be effectively employed in the study of the structure of various plant tissues, their arrangements, etc., which can shed light on the differences in component dietary fiber structures and their contribution to the texture of the fruit under consideration. Several microscopic techniques are employed to study the microstructure of plant cells and how it affects the food structure [64]. Microscopic examinations can show how the various nutrients like sugars, starch, polyphenols like condensed tannins, and other minor components like waxes are organized within and around the plant cells and tissue layers. It can reveal the micromorphology, anatomy, and ultrastructure of plant tissues, along with the location and occurrence of various biologically active components within them [64].

Light microscopy is usually employed to study the whole cell o tissue fragments, with or without staining. Different stains are employed to identify specific components in the case of non-pigmented plant parts. This allows for the identification and localization of various components within the plant tissue, and it gives an idea of

the spatial heterogeneity [105]. The magnification achieved in light microscopy is limited to the visual spectrum of light waves, and images are usually obtained at a magnification of up to 100x. Scanning electron microscopy (SEM) is a very convenient tool to study food structure as it combines the best features of light microscopy and transmission electron microscopy [106]. Scanning electron microscopy scans the surface of any solid sample, giving topographical and three-dimensional images at a resolution of less than 1 nm. The sample's external surface is scanned by an electron beam, processed, and shown as an image using a computer interface in black and white mode [107].

Chapter 3: Lignin is the Main Determinant of Total Dietary Fiber Differences Between Date Fruit (*Phoenix dactylifera* L.) Varieties

Article title: Lignin is the main determinant of total dietary fiber differences between date fruit (*Phoenix dactylifera* L.) varieties.

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3.1 Abstract

Date fruits (*Phoenix dactylifera*) of ten varieties, collected in the United Arab Emirates, were studied to determine their dietary fiber content and composition. Fourier transform infrared (FTIR) spectroscopy indicated that the dietary fiber components in all the date fruit varieties were similar. The major dietary fiber components, including cellulose, hemicellulosic components, lignin, and pectin, were analyzed by the Uppsala method. The total dietary fiber content in the date fruits analyzed (5.2%–8.4%) is comparable to commonly consumed dried fruits and is correlated with the content of lignin. The lignin was the main determinant of the total dietary fiber content in dates and its content was higher in semi-hard and hard fruit varieties.

3.2 Introduction

Meta-analyses of prospective epidemiological studies revealed that fruits and vegetables have moderate but significant associations with decreased risk of mortality and metabolic diseases including obesity, cardiovascular disease and some cancers [49, 108]. The consumption of fruits and vegetables are found to improve the overall diet profile by increasing the intakes of carbohydrates, dietary fiber, and micronutrient vitamins and minerals [48]. The dietary fiber is effective in potentiating health, e.g., by increasing fecal bulking and viscosity and decreasing the contact time of inflammatory agents and carcinogens and mucosal cells, enhancing the production of short chain fatty acids and the binding between bile acids and harmful dietary components such as estrogen and carcinogenic compounds, and by supplying antioxidants to the body [58]. The Institute of Medicine (IOM) set a fiber allowance

intake value of 14 g/1000 kcal, which is equivalent to 25 g/day for women and 38 g/day for men in the age range 19–50 years [61].

Dietary fiber is composed of various fractions including cellulose, non-cellulosic polysaccharides such as hemicelluloses, and non-carbohydrate fractions such as lignin. These are also the main structural components of the cell wall in plants. Dietary fibers can typically be classified as soluble or insoluble according to their water solubility [46]. Major dietary fiber fractions such as cellulose, hemicelluloses, and lignin are insoluble; they have beneficial effects on the human intestine [63]. The soluble dietary fiber fractions include non-cellulosic polysaccharides, oligosaccharides, pectin, and gums; they have beneficial effects on blood glucose and serum cholesterol levels [62, 109].

Date fruits represent a rich source of dietary fiber (6.5–11.5%), of which 84–94% is insoluble and 6–16% is soluble [2, 110]. Cellulose, hemicelluloses, and lignin make up the insoluble dietary fiber fractions in dates, whereas pectin, fructooligosaccharides, inulin, galactomannan, and β-glucan (among others) contribute to the soluble dietary fiber fractions. Some of these dietary fiber fractions will now be described in more detail. Cellulose is an unbranched linear chain of several thousand glucose units with β-1,4-glucosidic linkages. It is not digested to any extent by the enzymes of the human gastrointestinal system [111]. Hemicelluloses also contain backbones of glucose units with β-1,4 glucosidic linkages, but they differ from cellulose as they are smaller in size, are usually branched, and contain a variety of sugars, which primarily include xylose with some galactose, mannose, arabinose, and other sugars [26, 111]. Neither cellulose nor hemicelluloses, which together comprise the main constituents of insoluble dietary fiber in dates, are soluble in ethanol. Lignin

is a complex polymer containing about 40 oxygenated phenyl propane units including coniferyl, sinapyl, and *p*-coumaryl alcohols that have undergone a complex dehydrogenative polymerization. Due to strong intramolecular bonding, which includes carbon to carbon linkages, lignin is relatively inert. It also demonstrates a greater resistance to digestion than any other naturally occurring polymer. Finally, pectic substances are a complex group of polysaccharides in which D-galacturonic acid is a chief constituent. They are also structural components of plant cell walls and act as intercellular cementing substances. Pectin is highly water-soluble and is almost completely metabolized by colonic bacteria [111].

Date fruits, with their inherent polyphenols, are considered to be a nutritious and healthy source of dietary fiber with the added benefit of antioxidant activity [11]. Given the potential health benefits of dates, it is important to understand how various varieties of the fruit can contribute to human nutrition and, ultimately, well-being. Therefore, the aim of this study was to analyze the content of total dietary fiber in dates from ten Emirati varieties of dates (*Phoenix dactylifera*) collected from different locations in the United Arab Emirates (UAE).

3.3 Materials and methods

3.3.1 Samples

Fully mature (tamr stage) fruits of ten date (*P. dactylifera*) varieties were collected in 2017, each from three locations of the following in UAE (Al Saad, Al Foah, Bak Riya, Gummed, Al Dahid, and Wadi Al Khar). The ten studied varieties are presented in Figure 6.

Figure 6: The ten date varieties analyzed for dietary fiber composition in this study

3.3.2 Fourier transform infrared spectroscopy

The date fruit samples were placed directly into the sample holder of a Fourier transform infrared (FTIR) spectroscopy instrument (PerkinElmer Spectrum Two™ FT-IR spectrometer, USA). The absorbance was obtained after recording the background signal for the spectral range from 400 to 4000 cm^{-1} using Spectrum software.

3.3.3 Dietary fiber analysis

De-seeded fruit pieces (80 g) were mixed with cold, deionized water (160 mL) and grounded in a Sorwall Omni mixer until the sample was well homogenized. 0.5 g of the mixed sample was used for the analysis of dry matter (16 hours at 60°C in a vacuum oven). The dietary fiber content and composition were determined by the Uppsala method [86]. First, the simple sugars were washed and removed with 80% ethanol. Subsequently, the sample was treated with enzymes and the soluble dietary fibers were precipitated with 80% ethanol solution. Finally, the precipitated and insoluble polysaccharides were hydrolyzed with sulphuric acid. The released neutral sugars were quantitated as alditol acetates by gas-liquid chromatography (GC). Uronic

acids in the acid hydrolysate were determined spectrophotometrically after hydrolysis. Klason lignin was determined gravimetrically as ash-free acid-insoluble residue.

A spectrophotometric method was followed using an enzymatic assay kit (k-FRUC, Megazyme, Bray, Ireland) to determine the fructan (fructo-oligosaccharides and fructan polysaccharide) content with some modifications [112]. The extraction step was completed with a 0.5 g sample, which was accurately weighed into a glass tube with 10 mL of deionized water and kept at 80°C for 20 minutes. The filtration step was replaced by centrifugation: 1 mL of the mixture was centrifuged for 15 min at 10,600 g and 200 μL of the supernatant was then used for analysis. The samples were treated with α-galactosidase for the removal of raffinose-type oligosaccharides. This was completed before the degradation of maltosaccharides and sucrose, as detailed in the kit manufacturer's instructions. The sum of the dietary fiber from the Uppsala method and fructan was defined as the total dietary fiber (TDF).

3.3.4 Statistical analysis

Statistical analysis was completed using Minitab, version 19 (https://www.minitab.com/en-us/). One-way ANOVA was used to test for differences in dietary fiber content and composition among date fruit varieties. $p < 0.05$ was used as a measure of statistical significance.

3.4 Results and discussion

3.4.1 FTIR spectral fingerprints of date fruit fibers

The ten date fruit varieties studied here differed in their softness with Barhi and Lulu being soft varieties, Neghal and Dabbas being hard to semi-hard, and the rest of

varieties lie in between. In general, the fruits are composed of skin, sugary flesh, a distinct white part composed of fibrous bundles that is edible and devoid of sugars, and a seed [20]. The FTIR analyses of the edible parts of the fruits, showing peaks for various absorbances from 400 to 4000 cm^{-1}, are shown in Figure 7.

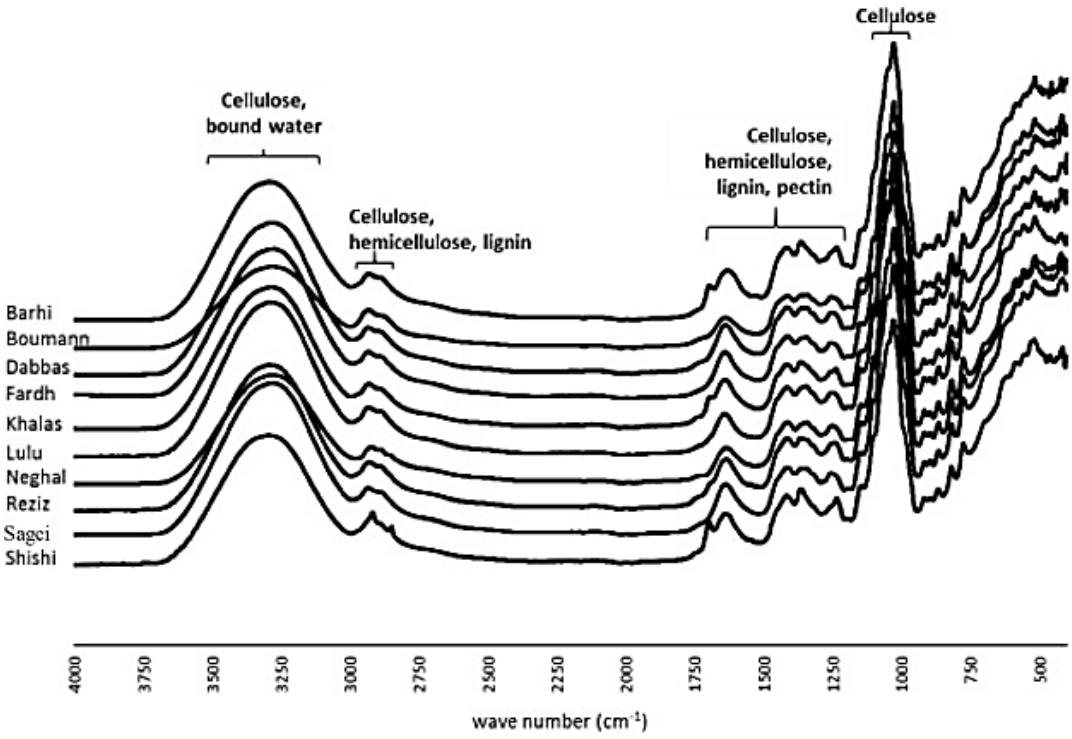

Figure 7: Fourier transform infrared absorbance spectra of ten Emirati date fruit varieties

All date fruits studied showed similar FTIR fingerprints, which suggests that they have comparable dietary fiber compositions. The spectra, interpreted according to Table 4, show the absorption bands associated with stretching and bending vibrations of the characteristic chemical groups of the various dietary fiber components including cellulose, hemicelluloses, lignin, and pectin [113-115]. The spectral fingerprints of the date fruit fibers reported here agree with those from previous studies of date fruit fibers [11].

Table 4: Peak positions and assignments of various chemical groups as evidenced from the Fourier transform infrared spectra of the UAE date fruit varieties

Wave number for a region in the spectrum (cm^{-1})	**Characteristic chemical groups and their behavior**	**Molecules responsible**
3600–3200	O-H stretching vibrations of the hydroxyl groups, inter- and intramolecular hydrogen bond vibrations	Cellulose, water molecule due to the moisture content
2960–2830	Stretching vibrations of the C-H of alkyl groups	Cellulose, lignin, and hemicellulose
1700–1500	C=C aromatic skeletal vibrations, arising from acetyl, and ester groups	Lignin, hemicelluloses
1022	C-O stretching of the pyranose ring	Cellulose
1740–1600	From various carbonyl groups, bending vibrations	Pectins bound water
1645–1612	C-O stretching of conjugated or aromatic ketones and in flavones	Lignin
1200–1400	C-O stretching, C-H stretching, C-H vibrations, CH2 bending, and asymmetric stretching mode vibration of methyl ester	Hemicelluloses, cellulose, pectin
1050–1040	C-O, C-C, and C-OH stretching vibrations, aromatic C-H in plane deformation	Cellulose, hemicelluloses, lignin, arabinose
1010–1018	C-O, C=C, and C-C-O stretching backbone vibrations	Galactomannans, pectins
910–750	C-H bending of syringyl units, aromatic ring; antisymmetric out-of-phase ring stretching	Hemicellulosic compounds

3.4.2 Total dietary fiber and its constituents in date fruits

The sugar residues, Klason lignin, uronic acids, and fructan components of the analyzed date fruits are presented in Table 5. The observed sugar residues resulting from the hydrolysis of the fruit polysaccharides, analyzed by GC, show that these dates consisted mainly of glucose, xylose, galactose along with limited amounts of arabinose and mannose with minute quantities of rhamnose and fucose. The quantity of uronic acid was usually lower in soft dates such as Barhi (0.7–0.8%), whereas Neghal and

Reziz had higher uronic acid values of around 1.1%. The average fructan content across varieties was 2–8% of the TDF with the soft variety Barhi having the highest fructan content (7.9% of TDF). The content of Klason lignin varied from around 25 to 40% of TDF in the samples. Lignin, the non-carbohydrate fraction of the dietary fiber, is the major dietary fiber component in the date fruits, which agrees with the findings of previous studies [11, 110]. The varieties Barhi, Lulu, and Khalas had low lignin contents while Sagei and Neghal had high lignin contents suggesting that the Klason lignin content increased with increasing fruit hardness.

The total dietary fiber (TDF) content found in the ten date varieties was 5.3–8.4% (Table 5) and the values are in accordance with results from previous studies [2, 26]. In general, the TDF content in dates is comparable to that of other dry fruits such as dried blueberries (7.5%), raisins (6.8%), apricots (7.3%), pears (6.4%), and cranberries (5.3%) but is much higher than that observed in fresh fruits [116]. According to the results, contribution of date fruits to the recommended daily intake of dietary fiber will vary according to variety. For example, three fruits of Barhi and Sagei, weighing approximately 13 and 35 g [117], will provide 2–3% and 7–10% of the recommended daily fiber intake, respectively.

The obtained neutral sugars are constituents of cellulose and hemicellulose. The pectin and lignin of the fruit cell walls contribute to the obtained values for uronic acids and Klason lignin. The glucose in date fruit polysaccharides comes mainly from cellulose and, to a lesser extent, from β-d-glucan [62]. According to literature, date fruit polysaccharides are mainly composed of cellulose, hemicelluloses (arabinoxylans and galactomannans), pectins (uronic acid derivatives), and lignin [11, 110]. Ishrud et al. isolated galactomannan from date seeds [22] and hence it is assumed that the fruits must also have the same polysaccharide. The values of the released neutral sugars

(galactose and mannose) in the dietary fiber analysis of these samples are thus added together and taken as galactomannan. Figure 8 shows that presumed levels of fructans, galactomannan, pectin, arabinoxylan, and cellulose/β-glucan did not differ much between the ten date varieties but the level of lignin differed considerably in these varieties and had a high significant correlation with the TDF content ($r = 0.964$, $p < 0.01$).

Table 5: Total dietary fiber (TDF) content and relative composition of fiber components (g/100 g dry weight) in UAE date fruit varieties

Variety	Sugar residues (% of dm)							Klason lignin	Uronic acids	Fructans	TDF
	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose				
Barhi	0.08±0.01	0.03±0.01	0.28±0.01	0.77±0.04	0.22±0.01	0.25±0.02	1.24±0.04	1.24±0.09	0.78±0.03	0.42±0.03	5.30
Boumann	0.08±0.01	0.03±0.00	0.29±0.01	0.80±0.11	0.21±0.00	0.52±0.04	1.27±0.07	1.93±0.10	0.82±0.08	0.13±0.01	6.09
Dabbas	0.08±0.01	0.03±0.00	0.27±0.02	0.88±0.04	0.23±0.01	0.58±0.04	1.33±0.05	2.94±0.24	0.97±0.01	0.24±0.06	7.56
Fardh	0.08±0.01	0.03±0.00	0.29±0.01	0.69±0.09	0.23±0.01	0.53±0.07	1.31±0.11	2.35±0.14	0.92±0.08	0.28±0.04	6.71
Khalas	0.11±0.02	0.03±0.00	0.34±0.04	0.77±0.13	0.24±0.02	0.32±0.03	1.46±0.23	1.64±0.27	0.93±0.16	0.36±0.08	6.20
Lulu Red	0.08±0.01	0.03±0.00	0.27±0.03	0.75±0.06	0.24±0.01	0.52±0.04	1.31±0.09	1.54±0.17	0.83±0.04	0.35±0.08	5.92
Neghal	0.10±0.00	0.03±0.01	0.31±0.01	1.11±0.03	0.21±0.00	0.53±0.02	1.54±0.02	3.18±0.32	1.12±0.01	0.28±0.09	8.42
Reziz	0.10±0.01	0.03±0.01	0.34±0.02	0.77±0.04	0.22±0.02	0.33±0.01	1.42±0.09	1.99±0.32	1.02±0.11	0.43±0.19	6.65
Sagei	0.09±0.00	0.03±0.00	0.29±0.02	0.77±0.08	0.21±0.01	0.68±0.03	1.25±0.07	3.00±0.44	0.81±0.22	0.30±0.05	7.43
Shishi	0.09±0.00	0.03±0.00	0.32±0.01	0.82±0.01	0.24±0.01	0.46±0.00	1.30±0.07	2.33±0.23	0.91±0.03	0.50±0.06	7.01

∗ Values of the different dietary fiber component represent the mean ± SD for three samples per variety. TDF is obtained by adding all the presented dietary fiber components

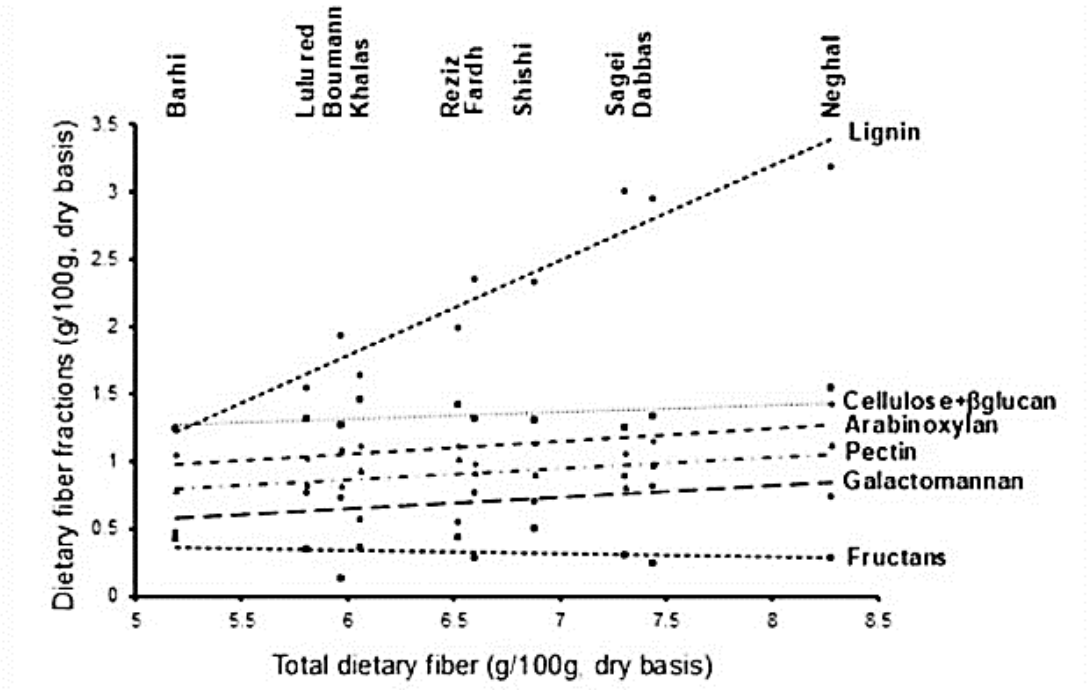

Figure 8: Scatter plot showing the relationships among total dietary fiber contents and the content of the different fiber fractions (mean values for three samples)

3.4.3 The variability in the fiber composition within the fruit tissues

Table 6 presents the differences in TDF and its components between the skin and the inner white bundles of the mesocarp in two date varieties, Fardh and Sagei. The TDF and especially lignin, arabinose and xylose contents are much higher in the skin and that the uronic acid content in the skin and the inner white part of the date fruits was more than twice that in the whole fruit. Date fruit skin had more than twice as much lignin than the whole fruit and that no lignin was detected in the inner white fiber bundles of the fruit. This variability in fiber components distribution is related to the types of cells constituting these tissues. The skin is rich in collenchyma cells in addition to sclereids, which are sclerenchyma cells highly thickened by lignin. In contrast, the mesocarp dominating the rest of the fruit is composed of parenchyma cells having thin primary cell walls mainly composed of cellulose [118].

Table 6: Dietary fiber in the skin and white parts of date fruits Fardh and Sagei (g/100 g dry matter)

Variety/ Sample	TDF	Cellulose + β-glucan	Arabinoxylan	Galactomannan	Lignin	Pectin
Skin (Mainly collenchyma cells & sclereids)						
Fardh	21.62	4.04	7.50	1.44	6.42	2.22
Sagei	18.66	3.28	6.77	1.51	5.28	1.82
Inner white fiber bundles of the mesocarp (parenchyma cells)						
Fardh	5.97	2.32	1.09	0.89	nd	1.67
Sagei	5.19	1.94	1.01	0.88	nd	1.36

TDF, total dietary fiber. nd, not detected.
Note: Values are means of the analysis of two samples. Analysis of fructans was not performed for these samples.

3.5 Conclusions

Analysis of ten UAE date varieties showed that date fruit is a good source of dietary fiber and that these varieties did not differ significantly in their constituent sugar residues or fructans. This study showed that lignin is the main determinant of the total dietary fiber content in date fruits and that harder date varieties have higher levels of lignin and total dietary fiber than softer varieties. It is suggested that date fruit by-products that remain after the extraction of sugars or syrups could be a promising source of dietary fiber and can be used as functional ingredients in a variety of foods such as ice cream, sausages, bakery products, and beverages to modify textural and emulsification properties [117, 119].

Chapter 4: Microscopic Investigations of Silicification and Lignification Suggest Their Coexistence in Tracheary Phytoliths in Date Fruits (*Phoenix dactylifera* L.)

Article title: Microscopic Investigations of Silicification and Lignification suggest their Coexistence in Tracheary Phytoliths in Date Fruits (*Phoenix dactylifera*, L.)

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4.1 Abstract

Date fruits are special representative of hard fruits and one of the richest sources of dietary silica and edible lignin, which are believed to have several health benefits. In this study, optical and scanning electron microscopy (SEM) were used to investigate the presence of associations between silicification and lignification in date fruits (*Phoenix dactylifera* L.). Phloroglucinol staining was employed to observe lignification in date fruits, while silicification was studied by SEM of whole fruits and their acid digesta. This work revealed the presence of heterogeneity and complexity in the silica phytoliths and the lignified structures in date fruits. It was found that lignin exists independently of silica in the secondary cell walls of parenchymal and sclereid cells and that silica exists independently of lignin in the spheroid phytoliths that surround the sclereid cells. Interestingly, a small proportion of lignin and silica seemed to co-exist as partners in the spiral coils of the tracheid phytoliths.

4.2 Introduction

Date fruits (*Phoenix dactylifera* Family: Palmae) are relatively dry fruits (moisture content, 10%–30%) with a high content of soluble sugars (60%–75%), accompanied by dietary fiber (5%–15%) and phenolic compounds (up to 5%) [10]. The dietary fiber composition of dates consists mostly of lignin, together with cellulose, hemicelluloses, and pectins [120]. Lignin is a class of high-molecular-weight phenolic polymers that provide rigidity to xylem vessels [78]. Date fruits are also rich sources of minerals, especially calcium, phosphorus, potassium, magnesium, iron, zinc, and cobalt [121]. In addition, a study reported that date fruits contained the highest concentration (0.02%) of dietary silica among 207 foods examined [122]. Dietary soluble silicon is believed to provide several health benefits to consumers,

including, *inter alia*, bone homeostasis and regeneration, stimulation of collagen synthesis, and skin, nail, and hair health [123, 124].

The study of silicification in the different organs and tissues of plants is an emerging field of research, e.g., regarding how and why plants assemble and use silicon. Biominerals such as silica are believed to provide plants with structural rigidity, mechanical strength, flexibility, as well as functional properties, including protection against biotic and abiotic stresses [88]. Silicon is absorbed by plants as water-soluble silicic acid ($Si(OH)_4$), which, at a concentration that exceeds its solubility (>2 mM), starts to deposit as colloidal amorphous hydrated silica ($SiO_2.xH_2O$) and/or dehydrated form(s) of condensed polysilicic acid ($SiO_x(OH)_{4-2x}$) [97]. Phytoliths are mineralized inorganic particles of silicon dioxide (SiO_2) that are precipitated from the soluble monosilicic acid that is absorbed by plants from silica-rich soils [100]. Silica supplementation in grasses leads to the impregnation of cell walls with silica particles, which improves the mechanical properties of their tissues and increases their tolerance to biotic and abiotic stresses [125].

Phytoliths are microscopic opal silica particles produced in many plants for variable functions [126]. Date palm tissues (roots, stems, and leaves) contain up to 1% silicon, which is found as aggregates or phytoliths that are mainly associated with the sclerenchyma of the vascular bundles; moreover, the possible association between lignification and silica deposition was investigated but was ruled out [104]. This study, suggests that silicification and lignification are associated in the xylem vessels but not in the sclereid cells of the skin or parenchymal cell walls of date fruits.

4.3 Materials and methods

4.3.1 Date fruits

Emirati date fruits at the mature, Tamr stage, were received from the Al Foah date company (Alsaad, Abu Dhabi, UAE). After being received, the fruits were stored frozen and were thawed for at least 3 hours before the experiments.

4.3.2 Chemicals and reagents

All chemicals and reagents used in this study were purchased from Sigma Aldrich (St. Louis, USA).

4.3.3 Light microscopy

The frozen dates were thawed, and samples of approximately 3 mm in length and breadth were cut from the fruits. The tissues were dipped in a graded series of ethanol (40%, 60%, and 80%) for 30 minutes each and finally kept in 80% ethanol overnight to enable the removal of sugars from the tissues and for fixation. The following morning, the pieces were washed again in 80% ethanol, followed by two washes in absolute ethanol and two washes (30 minutes and 1 hour) in xylene. The pieces were embedded in paraffin, and radial sections of the fruits were obtained using a rotary microtome. The sections were placed in glass slides and were double stained with safranin and Fast Green [127], as follows. Aqueous safranin (1%) was added to the sections on the slide for about 2 minutes. Excess dye was then washed off with tap water, and the slide was rinsed in deionized water. Counterstaining was performed using 0.5% Fast Green in 95% ethanol for 5 minutes, after which the slides were rinsed thoroughly in tap water and deionized water to remove the excess stain. After wiping

away the excess water, the slides were dried at 37°C in an oven for 30 minutes, the paraffin was removed from the sections in two changes of xylene (5 minutes each), and the dried sections were mounted with DPX. DPX - Dibutylphthalate Polystyrene Xylene, is a mixture of distyrene (a plasticizer) and xylene that is used as a synthetic resin-based mounting medium in microscopic studies (Sigma Aldrich, St. Louis, USA). For lignin observation, the sections were stained with 1% phloroglucinol in 92% ethanol for 3 minutes and then transferred to 25% hydrochloric acid. Once total reddening of the specimens was achieved, the sections were immediately mounted with DPX.

4.3.4 Scanning electron microscopy (SEM)

The dates were hand-cut into pieces of approximately 3 mm in length and breadth. The sugars were removed from the pieces by soaking in 80% ethanol (five times, 10 minutes each), and then dehydrated by two washes in acetone. The dehydrated pieces were mounted on aluminum studs using silver paint as an adhesive conductor. The pieces were sputter-coated with gold for observations. The SEM images of the fruit sections were obtained using an analytical scanning electron microscope (Jeol Analytical Scanning Electron Microscope, JEOL JSM-6010PLUS/LA, Tokyo, Japan). The scanning was performed at a low vacuum using a power of 20 kV, and the images were collected in the secondary electron imaging mode.

For microscopy of silica phytoliths, the date fruit pieces were digested in a mixture of equal volumes of concentrated sulphuric acid and nitric acid for at least 2 weeks. The resultant acid digesta were diluted with deionized water, and the precipitate was washed five times. The precipitate was then suspended in 95% ethanol and stored

in vials. Observation of silica was carried out by placing a drop of the sample on the aluminum stud of the SEM stage. The ethanol solution was allowed to evaporate, and the stud was mounted onto the stage of the SEM for image acquisition. SEM/energy-dispersive X-ray spectroscopy (EDS) was employed to obtain the elemental mapping of the date fruit phytoliths.

4.4 Results

4.4.1 The microstructure of date fruits

The microstructure of the edible part of the date fruit, as observed using optical microscopy, where various tissues take up different colors upon double staining with safranin and Fast Green is shown in Figure 9. Specifically, the lignin deposited in the sclereid cells and xylem vessels is stained with a pinkish-red color, while the tannins deposited in the vacuoles of the tanniferous layer are stained purple. Removal of the sugars from the fruit parenchymal cells leaves behind mainly the cell walls made of dietary fiber components (cellulose, hemicelluloses, and lignin), which are stained with a bluish-green color.

The date fruit exocarp (or skin) consists of one layer of epidermal cells covered by the cuticle and followed by two-to-three hypodermal cell layers and skin parenchymal layers. This is followed by an arrangement of distinct thick-walled sclerenchyma cells with a narrow cell lumen, called the stone cells or sclereids. The mesocarp, representing most of the fruit pulp and divided into outer and inner parts, consists of parenchymal cells and different levels of tanniferous middle layers in agreement with previous studies on date fruits [19]. The outer and inner mesocarp regions are separated by three-to-seven layers of tanniferous cells, mainly consisting of condensed polyphenols or tannins as reported before [19, 20]. Vascular bundles

consisting of xylem and phloem elements are scattered in the outer and inner mesocarp in various sizes. The chemical removal of water and sugars from the fruit might have caused certain losses of cellular structure due to cell wall rupture, especially in the soft varieties [128]. The lower part of the inner mesocarp is the inner white edible portion of the date fruit, which consists of fibrous cells that are devoid of sugar (Figure 9).

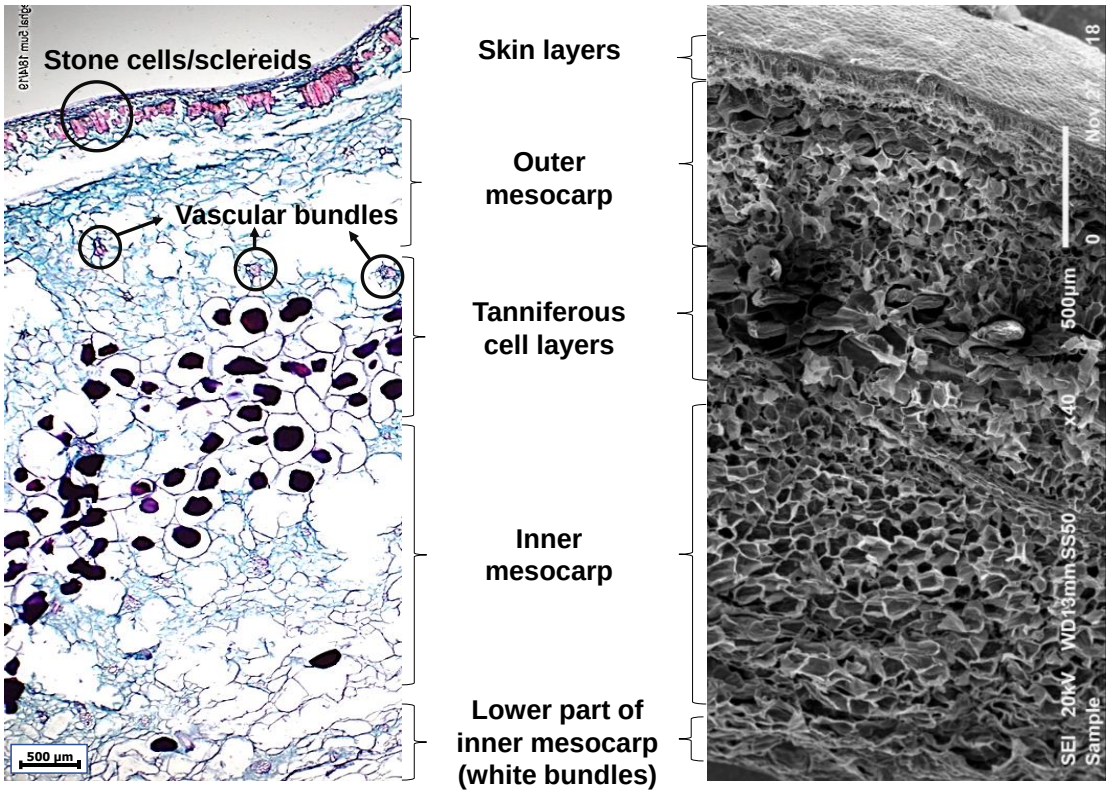

Figure 9: Microscopic images of mature date fruit showing cell layers from skin to inner mesocarp. (Left: Light microscopic image showing various cell layers in a mature date fruit (5 µm thick section) stained with safranin and Fast Green. The lignified cells (sclereids and xylem vessels) are stained red, non-lignified phloem elements and parenchyma are stained bluish-green, and tannins are stained purple. Right: Scanning electron microscopic image of a mature date fruit showing various cell layers).

4.4.2 Lignification of date fruits

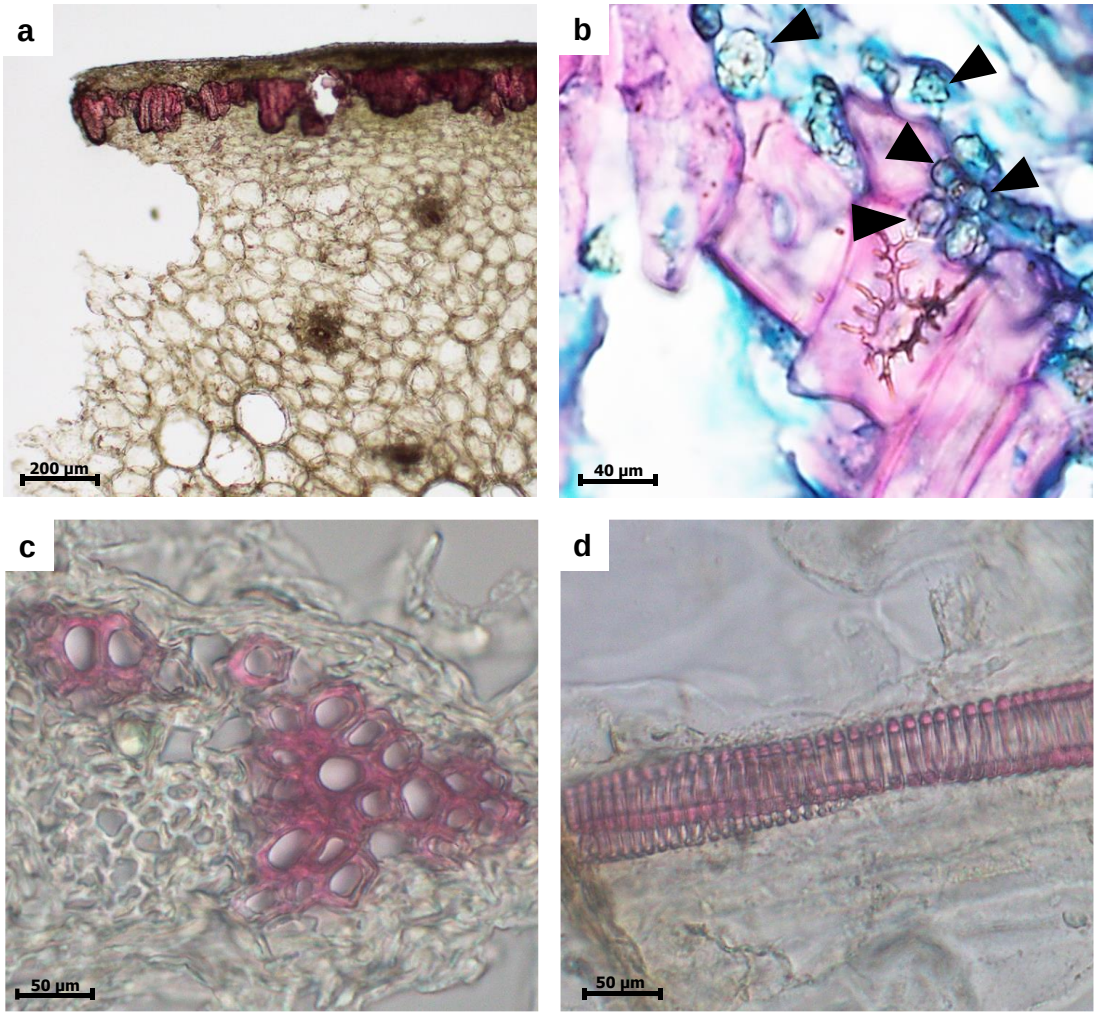

Figure 10: Light microscopic images of a 10-µm thick section of a date fruit stained with the lignin-specific phloroglucinol dye and showing lignin in bright red color. (a) Lignified sclereid cells (10×), (b) sclereid cells with secondary thickening of lignified cell walls surrounded by globular echinate phytoliths (100×) marked in the figure with black arrow heads, (c) xylem elements of the vascular bundles (40×), and (d) lignified xylem vessels (40×). Note: 3(b) is double stained with safranin and Fast Green and not by phloroglucinol.

Light microscopy sections of date fruits stained with lignin-specific phloroglucinol, which stains lignin with a bright-red color, demonstrated that lignin was deposited in various cells, including the sclereids, the xylem elements of the

vascular bundles, and the long xylem vessels of the mesocarp of the date fruit (Figure 10.a). The sclereid cells in the skin parenchymal layer are shown in Figure 10.b.

Figure 11 shows the SEM images of a mature date fruit section after the removal of sugars by ethanol washing, followed by dehydration with acetone. Figure 11.a presents the various cell layers in a date fruit from skin to inner mesocarp with many vascular bundles observed in the mesocarp region (marked in black circles). A vascular bundle, with an intact xylem element surrounded by the phloem within the sugar-free parenchymal cells is shown in Figure 11.b. Helical structures are clearly visible in Figure 11.c.

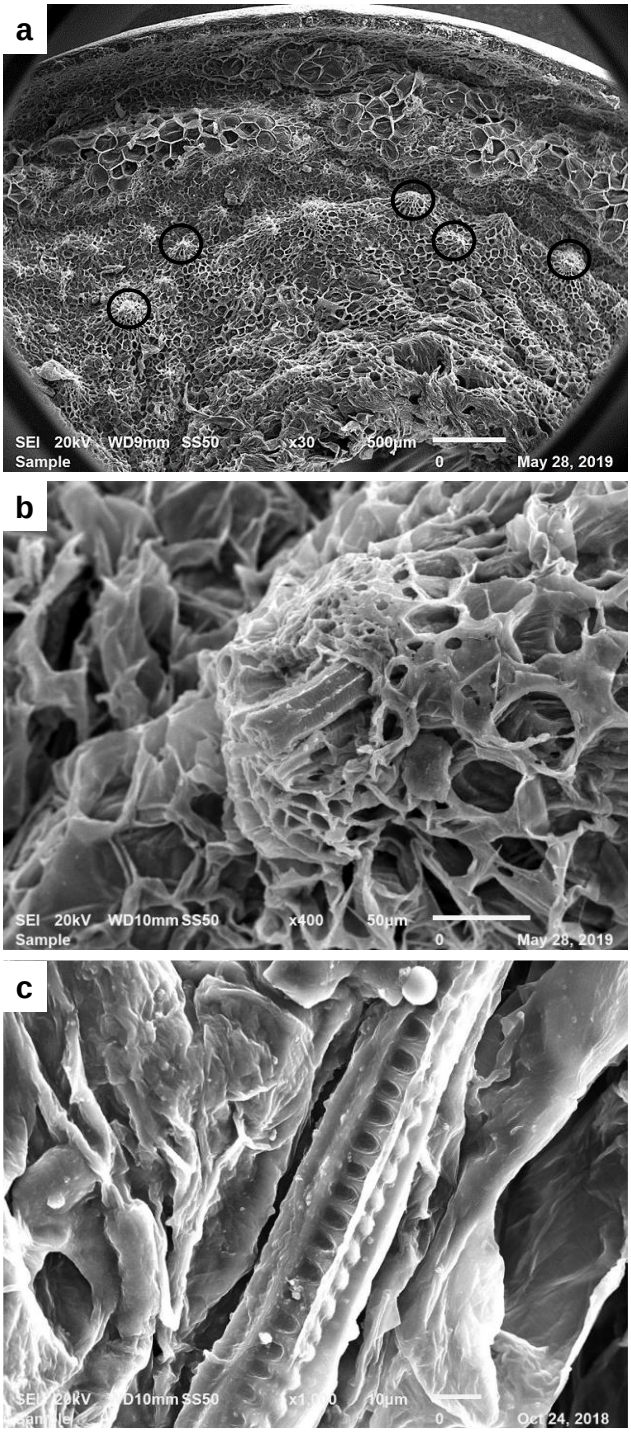

Figure 11: Scanning electron microscopic image of a mature date fruit showing (a) cell layers from skin to inner mesocarp (vascular bundles marked in black circles) (b) xylem of the vascular bundle among the parenchymal cells and (c) helical/spiral coils.

4.4.3 Silicification of date fruits

The digestion of date fruits in the concentrated acid mixture enables the removal of all digestible cell components leaving only silica and other ashes behind. Figure 10.b and Figure 12 depict the different manifestations of phytoliths, which are silicified plant bodies that were previously termed plant opals [129] in date fruits. Two types of phytoliths were observed, i.e., spheroid and tracheary phytoliths. The observed spheroid silica phytoliths were exhibiting different sizes (Figure 12.a). Moreover, they were echinate, with vivid, closely spaced petal-like projections that were arranged in a radiating fashion (Figure 12.b). These phytoliths were abundant around the sclereid cells within the skin layer (Figure 10.b). The second type of phytoliths observed in date fruits were tracheary annulate/helical phytoliths, which exhibited different shapes (Figure 12.c-f) and were part of xylem vessels in the mesocarp. They consisted of a silicified outer wall and a hollow lumen and were relatively straight, cylindrical, and elongated, with a consistent diameter. Sometimes, branched structures were observed that exhibited straight, rounded, or pointed ends. Tracheary phytoliths with different types of surface ornamentations or surface textures occurred as single structures or in articulated groups. They exhibited microporous structures, sometimes with minute open pores on their surface.

The elemental maps of the different phytoliths in acid digests of date fruits, i.e., spherical echinate and tracheary phytoliths, are depicted in Figure 13. These elemental maps show that the phytoliths are composed of silicon and oxygen in an atomic ratio of ca. 1:2, proving that the silica in phytoliths is deposited as SiO_2.

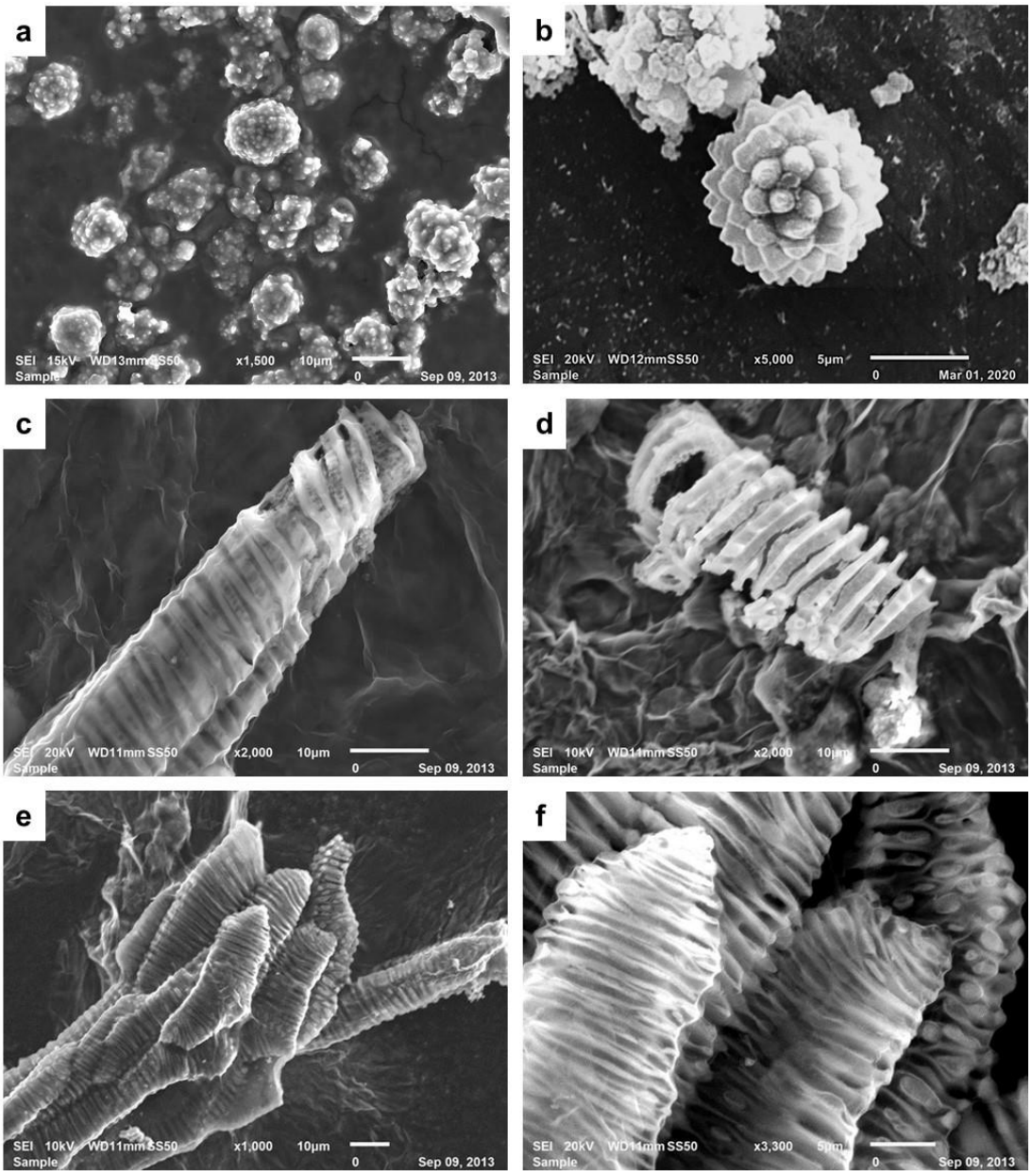

Figure 12: Scanning electron microscopic images of silica phytoliths (a, b) spheroid echinate phytoliths, (c, d) tracheary annulate/helical phytoliths as helical coils with varying patterns on the helices, (d) vivid porous surface on a silica helix, and (e, f) articulated tracheary pitted phytoliths showing oval to circular perforations on the surface.

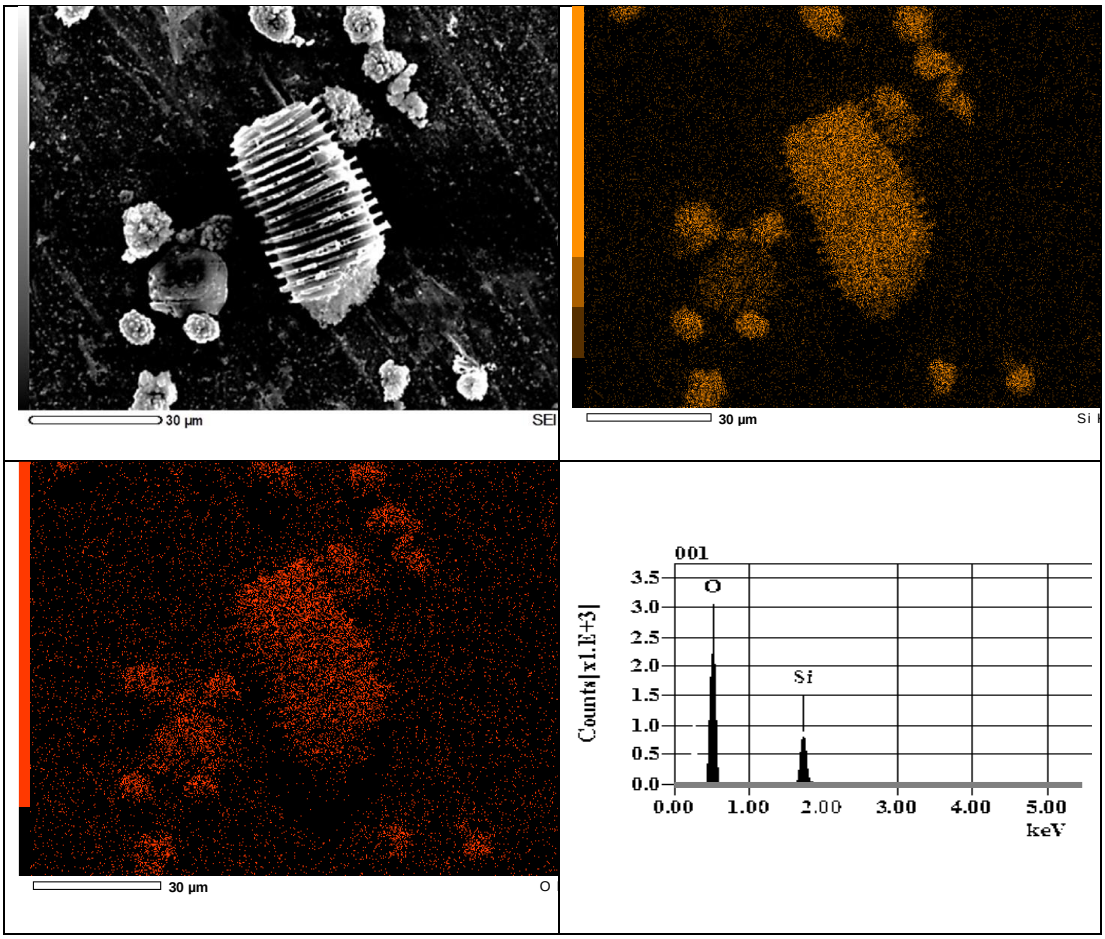

Figure 13: Scanning electron microscopy (SEM) /energy-dispersive X-Ray spectroscopy (EDS) images of silica phytoliths in date fruit.

The spheroid echinate and tracheary phytoliths obtained after digestion of a fruit sample with acid mixture contain only silicon (orange) and oxygen (red) in an atomic ratio of ca. 1:2, as shown by EDS mapping.

4.5 Discussion

In this study, optical and electron scanning microscopic techniques are used to assess the heterogeneity and complexity of the silicified and lignified structures within the microstructure of date fruits. The cell walls of parenchymal cells, the sclereids toward the skin of the fruit, and the walls of xylem vessels were highly lignified (Figure 9 and Figure 10). Lignification, which is a highly controlled mechanism that

starts at the early stages of tissue development, seems to play an important role in cell-wall thickening, thus providing rigidity, structural reinforcement, and flexibility to date fruits [130]. Lignins are mainly made of *p*-coumaryl, coniferyl, and sinapyl alcohols, which give rise to *p*-hydroxyphenyl (H), guaiacyl (G), and sinapyl alcohol (S) monomeric units, respectively [130]. Lignin is hydrophobic in nature, and it keeps the xylem vessels impermeable to water and assists in efficient transportation of water [78].

Although this has not been investigated further, date fruits were suggested to be the richest source of dietary silica [122, 131]. In this study, two types of phytoliths in date fruits are observed: spheroid phytoliths, which were concentrated mainly around the sclereid cells of the skin, and the tracheid phytoliths, located in the walls of the xylem vessels (Figure 10 and Figure 12). The spheroid phytoliths of date fruits, which exhibited a typical surface morphology ranging from warty to echinate/speculate, are typical of date palm species [93, 95, 104]. The spheroid echinate phytoliths, which were previously termed globular echinates [129], were abundant around the sclereid cells within the skin layers. The discovery of spheroid phytoliths in the teeth of Neanderthals in Iraq provided archaeological evidence that date fruits were part of their diet [14]. The existence of tracheid phytoliths in date fruits are presented in this study. Rajat et al. reviewed articles published for around 40 years until 2017, and reported the presence of tracheid phytolith-type in a few plants of the Asteraceae family, e.g., in the leaves of *Helianthus annuus*, and the stem and leaves of *Brachylaena*, *Vernonia*, and *Aspilia* species [132]. These types of phytoliths are commonly observed as multicelled silica skeletons with articulated structures in arid environments with high evapotranspiration rates [133]. The presence of silica in date

fruits may contribute to the structure of their cells and fruits by increasing water-use efficiency and resistance to biotic and abiotic stresses [88].

The similarity in spiral structure of lignin and silica phytoliths suggest their co-existence as components of the walls of the xylem vessels. The existence of a regulated association between silicification and lignification in plants has been suggested [101]. Organic carbon was found to be trapped within some phytoliths, which may reflect an important role of the chemical environment in silica deposition [134-136]. Studies in grasses suggested that lignification might be required for silica deposition [125, 137-139]. The number of phytoliths in date fruits is expected to be influenced by genetics (the cultivar) and agroclimatic conditions (soil, water supply, photosynthesis, etc.). The process of silicification is expected to be a hierarchical process that starts with the deposition of lignin on the hemicellulose–ferulic acid complex that is present in the secondary cell walls of plants. It was suggested that ferulic acid contributes to silicification *via* its attachment to the arabinoxylans that are present in grass cell walls and the anchoring of coniferyl alcohol, which initiates the deposition of lignin . It was also suggested that mixed-linkage glucans play a controlling role by preventing the interactions between $Si(OH)_4$ and arabinoxylan–ferulic acid complexes [125]. Conversely, the presence of silicon was suggested to have greater preference for organic polyhydroxyl compounds that are involved in lignin biosynthesis and to play a role in the production and accumulation of lignin in plants [103].

Here, several associations between lignin and silica depositions in date fruits were observed, namely the concentration of spheroid echinate phytoliths around the lignin-rich sclereid cells and the presence of possibly mixed spiral structures in the tracheary phytoliths in the xylem vessels. It is observed that the spiral shapes of the silica structures of the tracheary phytoliths in the xylem vessels mimicked those of

lignin, as visualized via specific staining within these vessels (Figure 10.d, Figure 11.b, and Figure 12.c–f). Lignin deposition follows the polymerization of phenolic monolignol radicals, which is catalyzed by localized oxidative coupling enzymes, mainly class III peroxidases and laccases [140].

This study emphasized the presence of lignin in the secondary walls of parenchyma cells and sclereid cells, as well as the presence of silica in two different phytolith morphologies. The results of the study suggest that silica and lignin coexist as partners in the lignified xylem tracheids. Further understanding the supramolecular networks formed by the structural organization of carbohydrates, lignin, and silica in date fruits and their roles in the mechanical strength, rigidity, and protection against biotic and abiotic stresses will unravel important biochemical features of this fruit. As both lignin and silica may contribute to the hardness of date fruits, the regulation of their levels in this fruit deserves further studies to identify their specific roles and the mechanism(s) that drive their accumulation.

Chapter 5: Dietary Fiber Components, Microstructure, and Texture of Date Fruits (*Phoenix dactylifera* L.)

Article title: Dietary Fiber Components, Microstructure, and Texture of Date Fruits (*Phoenix dactylifera,* L.)

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5.1 Abstract

Date fruits vary widely in the hardness of their edible part and they are classified accordingly into soft, semi-dry, and dry varieties. Fruit texture, a significant parameter in determining consumer acceptance, is related to the tissue structure and chemical composition of the fruit, mainly the ratio of sucrose to reducing sugars. This study aimed to understand the relationship between the chemical composition, microstructure, and texture profile of 10 major Emirati date fruits. The soluble sugars, glucose and fructose, represent ca 80 g/100 g of the fruits on the basis of dry weight (DW) while the dietary fiber contents varied 5.2–7.4 g/100 dg D.W. with lignin being the main determinant of the variability. The textures of the samples were studied using instrumental texture profile analysis. While no correlation was found between the soluble sugar and texture parameters in this study, the different fiber constituents correlated variably with the different parameters of date fruit texture. Lignin, arabinoxylan, galactomannan, and pectin were found to correlate significantly with fruit hardness and the related parameters, gumminess and chewiness. Both lignin and arabinoxylan correlated with resilience, and arabinoxylan exhibited a strong correlation with cohesiveness.

5.2 Introduction

Texture is a useful quality to use for determining the consumer acceptance of fruits and their utilization for various processing operations. Bourne (2002) defined the textural properties of a food as “the group of physical characteristics that arise from the structural elements of the food that are sensed primarily by the feelings of touch, are related to deformation, disintegration and flow of food under force, and are measured objectively by functions of mass, time, and distance” [28]. Perceived texture

is closely related to the structure and composition of foods at both microscopic and macroscopic levels. Thus, fruit texture is influenced by the chemical composition and cellular constitution of the fruit.

Date fruits at full maturity Tamr stage are primarily composed of sugars (60–80%) with the rest of the weight being moisture (10–30%), dietary fiber (5–12%), phenolic compounds (up to 4%), and other minor constituents on a fresh weight basis [10]. As they are variable in their moisture contents, date fruits are classified as soft (>30% moisture), semi-dry (20–30% moisture), and dry (<20% moisture) varieties on the basis of their moisture content. The types of sugar upon harvest, especially the sucrose to reducing sugars ratio, also plays an important role in classification of date fruits [31, 32]. Most of the varieties grown in the United Arab Emirates and neighboring countries are soft and semi-dry dates. These varieties are mostly related despite some differences imposed by genetics, cultivation, and environmental factors [31, 33]. In addition, differences in the content of soluble and insoluble dietary fibers contribute considerably to the variation between varieties [34, 141], and the varying levels of phenolic compounds contribute to the color and possibly texture of the fruit [35, 142]. It has been suggested that texture of date fruit depends on anatomical cell wall structure, specifically skin cell size and shape of the underlying pericarp tissue layers [36, 37]. In addition, it has been shown that the nanostructure of pectin, hemicellulose, and cellulose in cell walls affects the texture and firmness of pears [30]. Microscopic analysis, such as light microscopy, can be employed to identify different tissue structures, such as phenolic compounds in the exocarp and mesocarp [142]. Moreover, scanning electron microscopy can be employed to study the structure and distribution of dietary fibers [143]. Another useful technique for analyzing food properties is texture profile analysis (TPA), which assesses food texture [144, 145].

Through a double-bite compression test, TPA provides insight into how samples behave when they are chewed, and it quantifies multiple textural parameters, including hardness, cohesiveness, springiness, and resilience. A previous study presented substantial variability in the physical properties and texture profiles of fruits from 21 Emirati date varieties [146]. Understanding the texture profiles requires relating them to chemical composition, especially sugars and fibers, which are believed to influence texture, particularly hardness, elasticity, and stickiness.

This study aimed to understand the relationship between the chemical composition and microstructure of ten major Emirati date fruit varieties and their texture profile. Fruit carbohydrates, including the soluble sugars (glucose and fructose) and dietary fiber components (cellulose, hemicelluloses, pectin, and fructans) were determined and studied in terms of their distribution in different fruit tissues/cells and their correlation with texture parameters, including hardness, adhesiveness, cohesiveness, gumminess, chewiness, springiness, and resilience. The findings of the current study provide a better understanding of the sensory preference for different date fruits and can also be applied in date processing sector.

5.3 Materials and methods

5.3.1 Date fruits

Tree samples of ten different Emirati date fruits varieties at the mature (Tamr) stage, were received from Al Foah date factory (Al Saad, Abu Dhabi, UAE): Barhi, Boumann, Dabbas, Fardh, Khalas, Lulu red, Neghal, Reziz, Sagei, and Shishi.

5.3.2 Analysis of fiber components and soluble sugars

Dietary fiber components were analyzed using the Uppsala method, as described previously [86, 120]. For the analysis of soluble sugars, 1 g of date fruit samples were weighed in falcon tubes, homogenized using an Ultra-Turrax homogenizer, and extracted four times using 10 mL of 0.1% orthophosphoric acid and intermittent centrifugation at 4600 rpm for 15 minutes at 4°C. The supernatants were pooled in 50 mL volumetric flasks, and the volume was adjusted. Then, the samples were filtered through a 0.45 μm filter membrane into vials and analyzed via high performance liquid chromatography. Separations were conducted on a 300 mm-long μ-Bondapak NH2-column (Waters Corporation, Milford, Massachusetts, USA) with an internal diameter of 3.9 mm and particle size of 10 mm using 83:17 (v/v) acetonitrile/water as the mobile phase at a flow rate of 1.5 mL/minute. Peaks were detected using a diode array detector at 190 nm and quantified against authentic standards of glucose and fructose (Sigma-Aldrich Corporation, Darmstadt, Germany).

5.3.3 Light microscopy

Hand-cut sections of immature dates were stained with Mayer's hematoxylin (HiMedia, Kennett Square, Pennsylvania, USA). The commercially available pre-prepared stain was added to sections placed on glass slides and retained for 40 to 60 seconds. Excess stain was washed of using de-ionized water, a cover slip was added, and a microscope was used to observe the sections in aqueous media. Double staining with Safranin and Fast Green was also used to observe the tissues of mature date fruits. Before the observation of mature date fruits, frozen dates were thawed, cut into pieces of ~3 mm in length and breadth, and dipped in ethanol in a series of 40%, 60%, and 80% for 30 minutes each and finally kept in 80% ethanol overnight for

dehydration and removal of sugars. The next morning, the pieces were washed again in 80% ethanol, followed by two washes in absolute ethanol and two washes for 30 minutes and 1 hour in xylene. The pieces were then embedded in paraffin, and radial sections of the fruits were prepared using a rotary microtome. The sections were progressively rehydrated, stained, and then dehydrated before observing them (mounted in a synthetic resin-based mounting medium), according to the procedure previously demonstrated [127].

5.3.4 Scanning electron microscopy

Date fruit pieces of ~3 mm in length and breadth were freed from the sugars by soaking them in 80% ethanol five times for 10 minutes, and then, they were dehydrated by washing twice with acetone. Next, the dehydrated pieces were mounted on aluminum studs using silver paint as an adhesive and conductor. The pieces were then sputter-coated with gold before observations. The scanning electron microscopy images of the fruit sections were obtained using an analytical scanning electron microscope (Jeol Analytical Scanning Electron Microscope, JEOL JSM-6010PLUS/LA, Tokyo, Japan). Scanning was conducted in a low vacuum using a power of 20 kV, and the images were collected in secondary electron imaging mode.

5.3.5 Instrumental texture profile analysis

Instrumental TPA attributes were measured using a computerized CT3 texture analyzer equipped with a 4.5 kg load cell (TA instruments, Middleboro, MA, USA), which generates plots of force (g) versus time (s). A 2-mm-diameter penetration probe was used to measure the textural profile of the date fruit samples in the two-compression cycle model. All experiments were conducted at 25°C ± 2°C). One pitted

date was divided into two equal halves, one side was placed over the other, and the middle of the date halves was penetrated at a 5 mm target value at a compression rate of 1 mm/s. The location of the middle of the date halves was selected for penetration on the basis of previous trials. All measurements were performed in 15 replications. The texture parameters of hardness cycles 1 and 2, adhesiveness, cohesiveness, gumminess, chewiness, and resilience were calculated using the built-in software program.

5.4 Results

The carbohydrate composition of Emirati date fruits. Fruits from ten Emirati date fruit varieties were used to investigate the relationship between their carbohydrate composition, microstructure, and texture. Table 7 presents the percentage of soluble sugars and dietary fiber components in the fruits on dry weight basis. These date fruits were dominated by the soluble sugars glucose and fructose that represented 38.2–44.7% and 36.8–40.1% of the total dry mass, respectively. The contents of total dietary fiber in these varieties varied 5.3–8.4% with the primary component being lignin (1.2–3.2%) [120]. A considerable variation was also observed in the other dietary fiber components like fructans (0.1–0.5%) and other neutral sugar from gas chromatography (GC) values of dietary fibers such as glucose (1.2–1.5%), arabinose (0.3%), xylose (0.7–1.1%), galactose (0.3–0.7%), mannose (0.2%), and uronic acids (0.8–1.1%) constituting cellulose/β-glucan (1.2–1.5%), arabinoxylan (1–1.4%), and galactomannan (0.5–0.9%). The order of varieties in terms of dietary fiber content was Neghal > Dabbas > Sagei > Shishi > Fardh > Reziz > Khalas > Boumann > Lulu red > Barhi, which was related to their hardness (see below).

Table 7: Contents of soluble sugars, total dietary fiber, and dietary fiber components in 10 Emirati date fruit varieties (g/100 g dry weight)

Variety	Soluble Sugars		Total Dietary Fiber	Dietary Fiber Component					
	Glucose	Fructose		Fructans	Cellulose+β-Glucan	Arabinoxylan	Galactomannan	Lignin	Pectin
Barhi	44.7 ±3.1^{b}	38.3±1.7^{a}	5.19 ±0.23^{e}	0.42 ± 0.03^{a,b}	1.24 ± 0.04^{b}	1.05 ± 0.04^{b}	0.47 ± 0.02^{c}	1.24 ± 0.09^{e}	0.78 ± 0.03^{c}
Boumann	39.0±1.3^{ab}	36.8±0.3 ^{a}	5.97± 0.25^{d,e}	0.13 ± 0.01^{c}	1.27 ± 0.07^{a,b}	1.09 ± 0.12^{b}	0.73 ± 0.04^{b}	1.93 ± 0.10^{c,d,e}	0.82 ± .08^{b,c}
Dabbas	40.9±2.2 ^{ab}	37.9±0.9 ^{a}	7.44± 0.40^{a,b}	0.24 ± 0.06^{b,c}	1.33 ± 0.05^{a,b}	1.15 ± 0.05^{b}	0.81 ± 0.05^{a,b}	2.94 ± 0.24^{a,b}	0.97 ± 0.01^{a,b,c}
Fardh	41.3±1.9 ^{ab}	38.9±0.4 ^{a}	6.59±0.25^{b,c,d}	0.28± 0.04^{a,b,c}	1.31 ± 0.11^{a,b}	0.98 ± 0.10^{b}	0.76 ± 0.07^{b}	2.35 ± 0.14^{b,c}	0.92 ± 0.08^{a,b,c}
Khalas	41.7±0.9 ^{ab}	40.1±0.5 ^{a}	6.06±0.74^{c,d,e}	0.36± 0.08^{a,b,c}	1.46 ± 0.23^{a,b}	1.11 ± 0.16^{b}	0.56 ± 0.05^{c}	1.64 ± 0.27^{c,d,e}	0.93 ± 0.16^{a,b,c}
Lulu red	42.1±0.6 ^{ab}	39.8±0.9 ^{a}	5.81 ± 0.38^{d,e}	0.35± 0.08^{a,b,c}	1.31 ± 0.09^{a,b}	1.02 ± 0.09^{b}	0.76 ± 0.05^{b}	1.54 ± 0.17^{d,e}	0.83 ± 0.04^{b,c}
Neghal	38.2±2.4^{a}	39.4±1.6 ^{a}	8.29 ± 0.23^{a}	0.28± 0.09^{a,b,c}	1.54 ± 0.02^{a}	1.43 ± 0.04^{a}	0.74 ± 0.01^{b}	3.18 ± 0.32^{a}	1.12 ± 0.01^{a}
Reziz	39.4±1.6 ^{ab}	37.6±2.1 ^{a}	6.52±0.45^{b,c,d}	0.43 ± 0.19^{a,b}	1.42 ± 0.09^{a,b}	1.11 ± 0.05^{b}	0.55 ± 0.02^{c}	1.99 ± 0.32^{c,d}	1.02 ± 0.11^{a,b}
Sagei	41.6±1.4 ^{ab}	37.1±0.7 ^{a}	7.31±0.67^{a,b,c}	0.30± 0.05^{a,b,c}	1.25 ± 0.07^{a,b}	1.06 ± 0.09^{b}	0.89 ± 0.04^{a}	3.00 ± 0.44^{a,b}	0.81 ± 0.02^{c}
Shishi	41.3±1.1 ^{ab}	38.3±0.6 ^{a}	6.89±0.39^{b,c,d}	0.50 ± 0.06^{a}	1.30 ± 0.07^{a,b}	1.13 ± 0.02^{b}	0.70 ± 0.02^{b}	2.33 ± 0.23^{b,c}	0.91 ± 0.03^{a,b,c}
Range	38.2-44.7	36.8-40.1	5.2-7.4	0.13-0.50	1.24-1.54	0.98-1.43	0.47-0.89	1.24-3.18	0.81-1.12

The value for each variety is the average of three samples collected at different locations in the UAE. The mean values ± standard deviation with different superscript letters within each column are statistically different ($p < 0.001$)

5.4.1 The microstructure of date fruits

Figure 14 presents the microstructure of a date fruit (var. Sagei), at Kirmi stage (75 days after fruit set), stained with Mayer's hematoxylin and observed via optical microscopy. The edible part of the date fruit, which is the flesh or pericarp, consisted of three distinguishable tissues: exocarp, mesocarp, and endocarp.

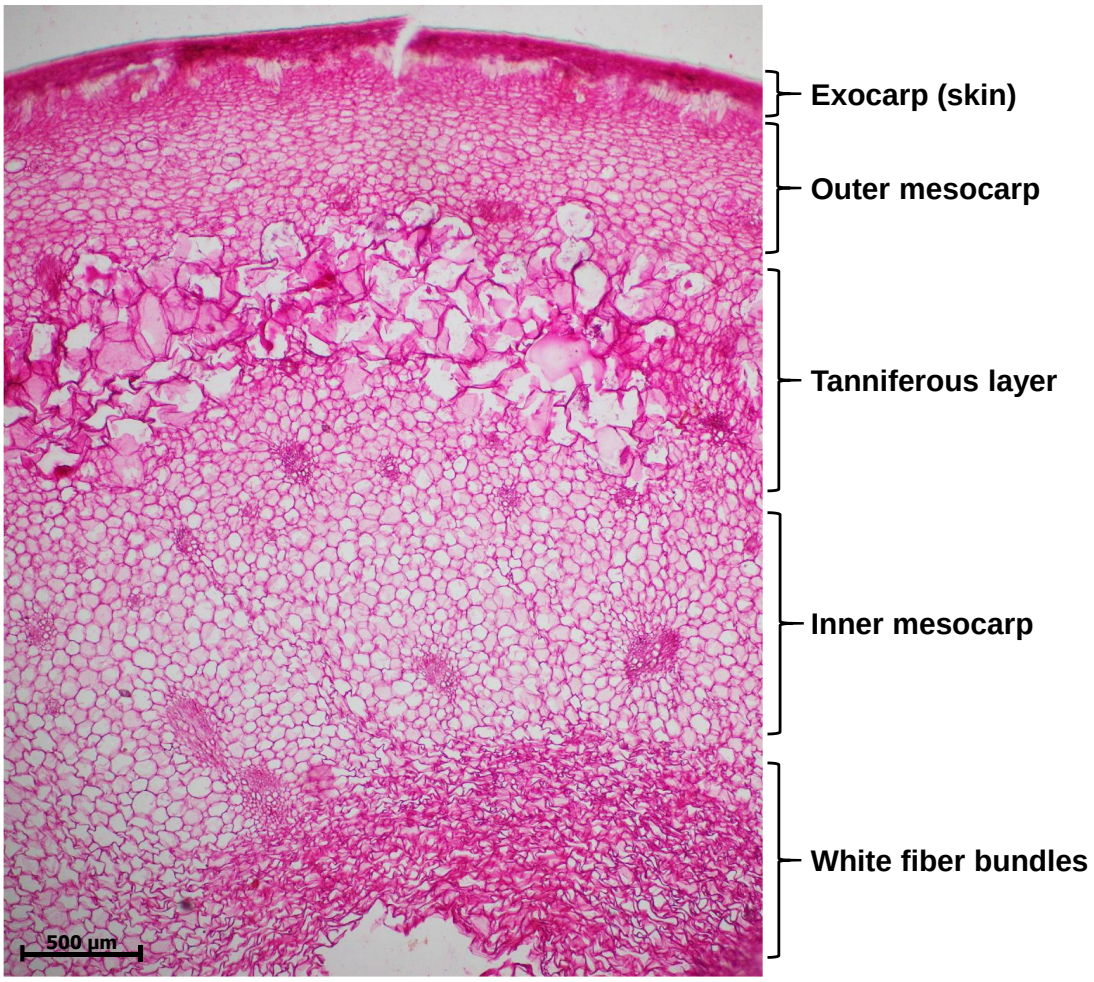

Figure 14: Light microscopy image of a date fruit specimen with a 5-µm thickness and viewed at a magnification of 4× stained with Mayer's hematoxylin (exhibiting the different tissues of the fruit: (1) the exocarp, (2) the outer mesocarp, (3) the tanniferous layer, (4) the inner mesocarp, and (5) the innermost white fiber bundles of the inner mesocarp).

The variability in the microstructure in selected varieties is presented in Figure 15. The portion in between the flesh and the seed coat was a distinct white part, the lower mesocarp, which was composed of fibrous bundles that are edible and devoid of sugars (Figure 17). The thickness and nature of the white fibrous layers, which were adhered to the flesh of the fruit forming the lower mesocarp region, differed between the date fruit varieties.

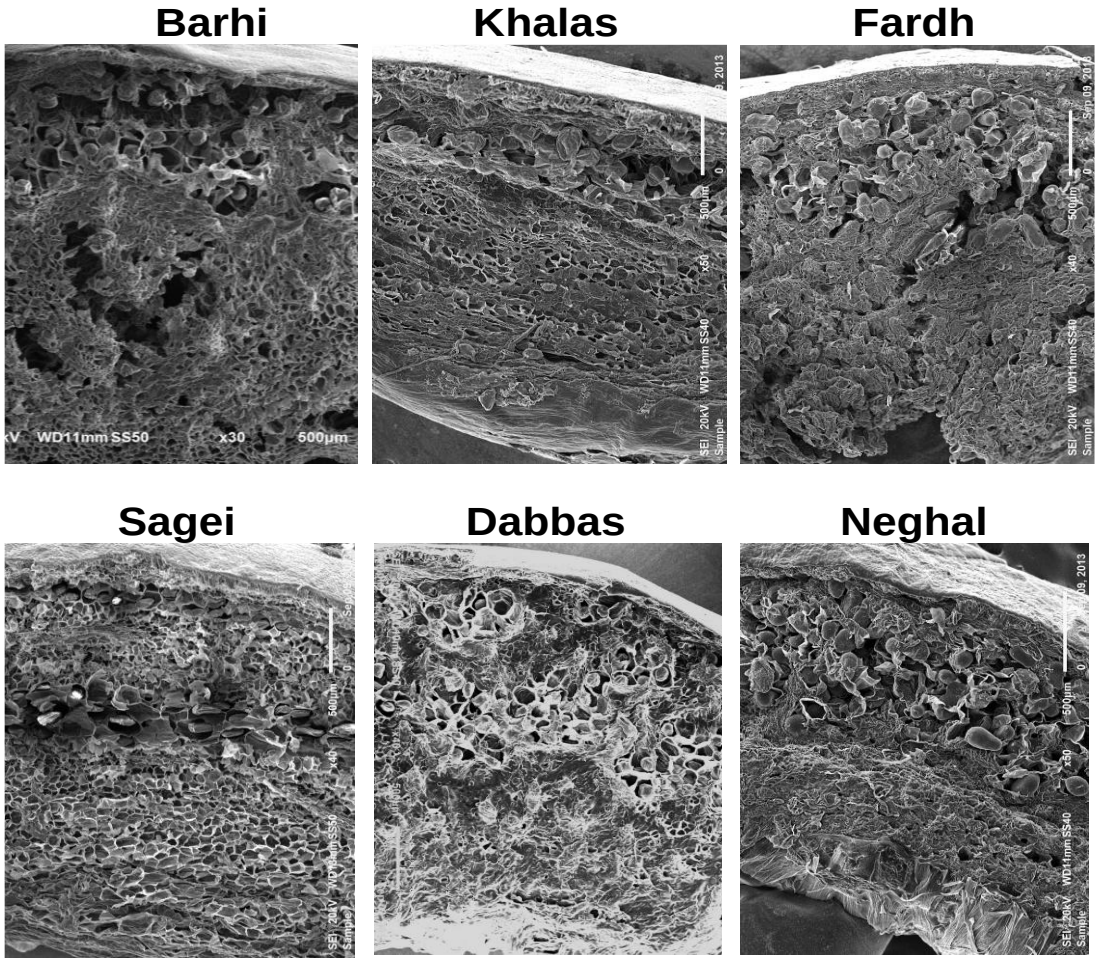

Figure 15: Variability of the microstructure of selected date varieties.

The exocarp, or epicarp (Figure 16.a), consisted of five distinct layers: a non-cellular cuticle (Figure 16.b & c), an epidermal layer (Figure 16.d), a hypodermis (Figure 16.d), a skin parenchyma layer (Figure 16.e), and a layer of sclereid cells

(Figure 16.f). A dense layer of epicuticular wax covered the surface of the fruit, except for the region close to the perianth. The single-cell epidermal layer is composed of small, elongated cells with cellulosic cell walls. Below the epidermis is the hypodermis, composed of one or two layers of tangentially elongated collenchyma cells. In some varieties, there was a layer of parenchyma cells, which are called the parenchyma of the skin. The hypodermis primarily consisted of collenchyma cells and was followed by highly lignified sclereid (or stone) cells. These sclereid cells, which represent the last layer of the skin, were elongated and radially oriented, with the long axis parallel to the fruit radius.

The transition from the exocarp to the mesocarp varied between varieties, depending on the nature of the outer mesocarp and the tanniferous layer (Figure 16.g-h). The fruit mesocarp consisted of parenchyma cells organized into two morphologically distinct zones intermediated by 3–7 layers of tanniferous cells, which consist primarily of condensed polyphenols or tannins [19, 20]. The inner mesocarp consisted of larger polyhedral parenchyma cells, which formed a spongy tissue and stored soluble sugars (Figure 16.h-i). At maturity, the mesocarp is not in direct contact with the seeds. In immature fruits, biomineralized calcium oxalate deposits occur as needlelike structures with pointed ends (Figure 16.j), which are called raphides. The specialized cell structures that contain them are called idioblasts (Figure 16.k) [88, 91]. Vascular bundles (Figure 16.l), consisting of xylem and phloem elements, were scattered in various sizes all over the mesocarp with less abundance towards the interior.

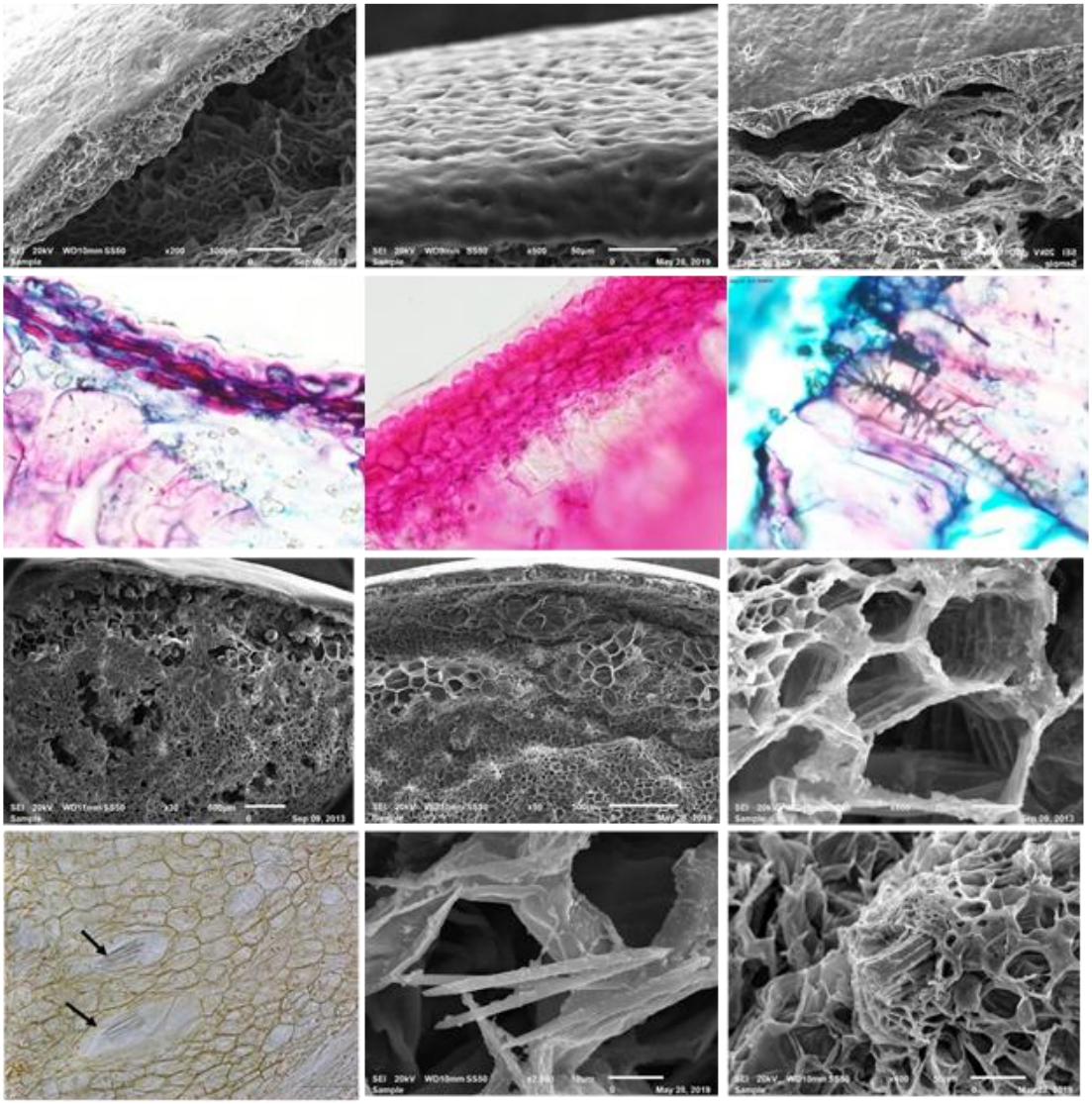

Figure 16: Micrographs of date fruit from a scanning electron microscope (a–c, g–i, k–l) and light microscope (d–f, j).
(a) The covering cuticle layer; (b) the cuticle, epidermis, hypodermis, and sclereid cells separating from the mesocarp; (c) image focusing on the sclereid cells at the last cell layer of the skin; (d) the cuticle, epidermis, hypodermis, and sclereid cells (stained light pink); (e) enlarged image showing the single hypodermal cell layer, a two-cell hypodermal layer, and part of the sclereid layer surrounded by phytoliths; (f) an enlarged sclereid cell showing the secondary cell walls thickened by lignin; (g) the transition from the exocarp to the mesocarp and (h) the tanniferous layer; (i) enlarged parenchyma cells of variable sizes and shapes; (j) light microscopy images showing idioblasts containing calcium oxalate needles in unstained specimen; (k) enlarged idioblasts showing calcium oxalate needles; and (l) an enlarged xylem vessels showing an emerging tracheid.

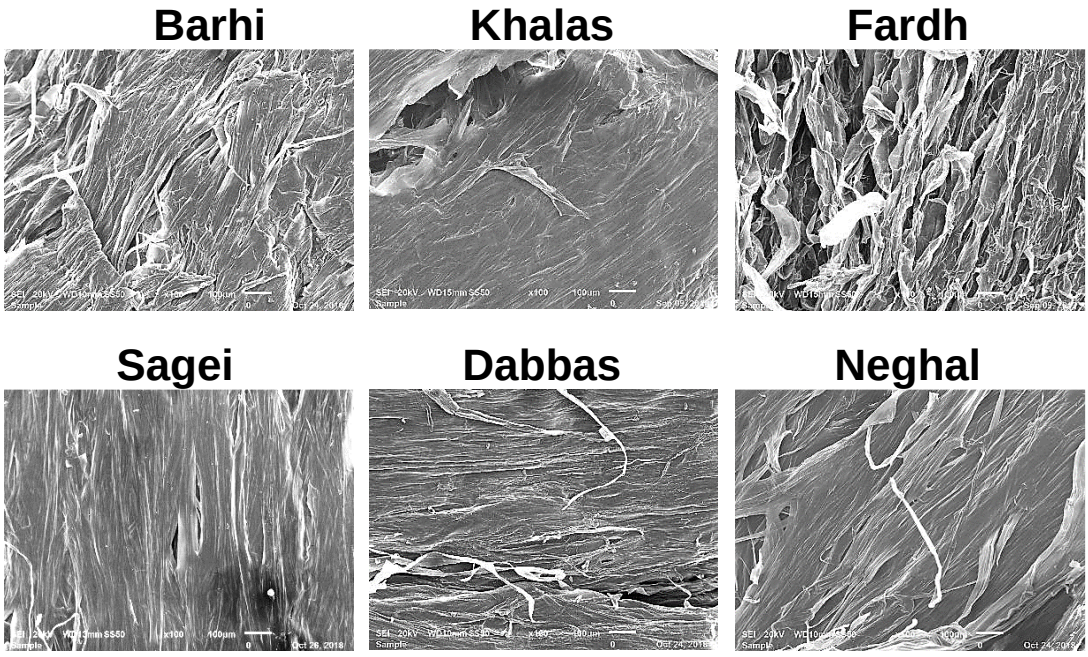

Figure 17: The internal white fiber bundles terminal to the mesocarp of selected date fruits.

5.4.2 Instrumental texture profile analysis

The instrumental TPA attributes were determined from the force–time curves presented and explained in Figure 18. The ten date varieties studied here differed in their textural properties (Figure 20). For example, Barhi and Lulu were found to be soft varieties, Neghal and Dabbas were found to be hard to semi-hard in texture, and Khalas and Fardh fell in between these. Table 8 presents the Pearson correlation coefficients between texture parameters, soluble sugars, and dietary fiber components. No correlation was found between the soluble sugars and texture parameters. Lignin and total dietary fiber were highly correlated with hardness, gumminess, chewiness, and resilience, whereas all other correlations were moderate. There were correlations between arabinoxylan and hardness, gumminess, chewiness, cohesiveness, and resilience; between galactomannan and hardness; between fructans and springiness and adhesiveness; and between cellulose+β-glucan and gumminess. In addition, no

correlation is found between the silica content in the date fruits and their texture properties (results not shown).

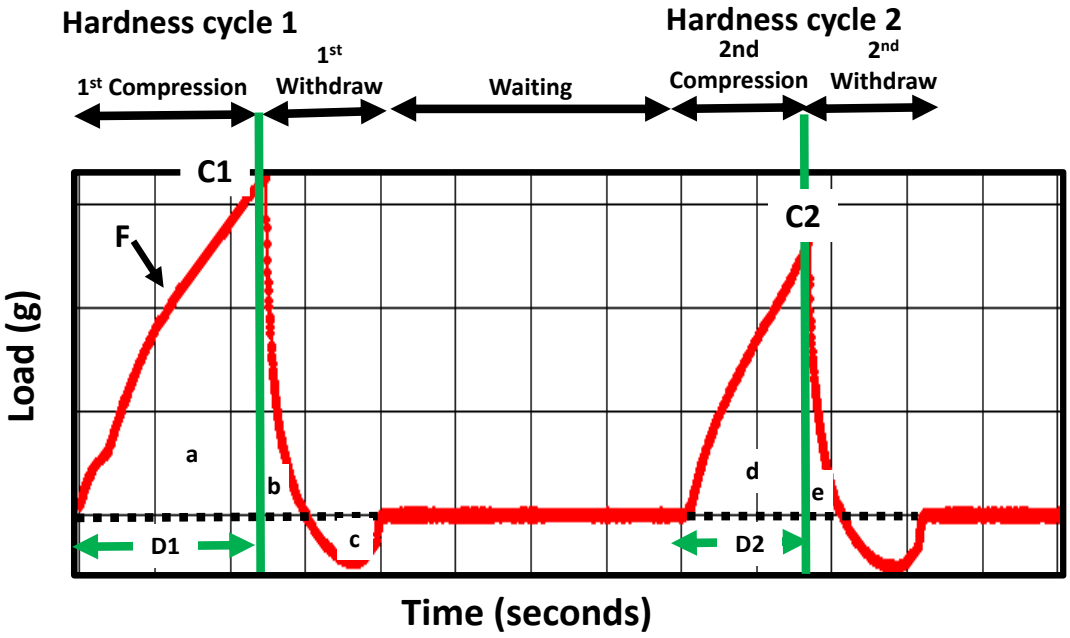

Figure 18: A typical texture profile of date fruits.

The analysis is based on two-compression cycles mimicking bites with teeth. Hardness cycles 1 and 2 represent the forces required for the penetration of the food material during bite 1 and bite 2, and they are measured by the heights C1 and C2, respectively. Fracturability (F) is a measure of brittleness. Adhesiveness, a measure of stickiness to surfaces, is defined as the force necessary to pull the compressing probe out of the sample, and it is measured as the area above the curve for the first negative peak (c). Cohesiveness, or consistency, measures the internal bonds keeping the product intact and is calculated as (d+e)/(a+b). Springiness is the extent to which a deformed material returns to its initial condition and is calculated as D2/D1*100. Gumminess is the energy required to break a semisolid food into fragments, and it is calculated as the product of hardness and cohesiveness. Chewiness is the energy

required to convert a solid food into a softer state suitable for consumption, and it is calculated as the product of hardness, cohesiveness, and springiness. Resilience describes the ability of the sample to return to its original form after being compressed, and it is calculated as the area under the curve after the peak force is reached divided by the area under the curve before the peak force is reached, i.e., b/a.

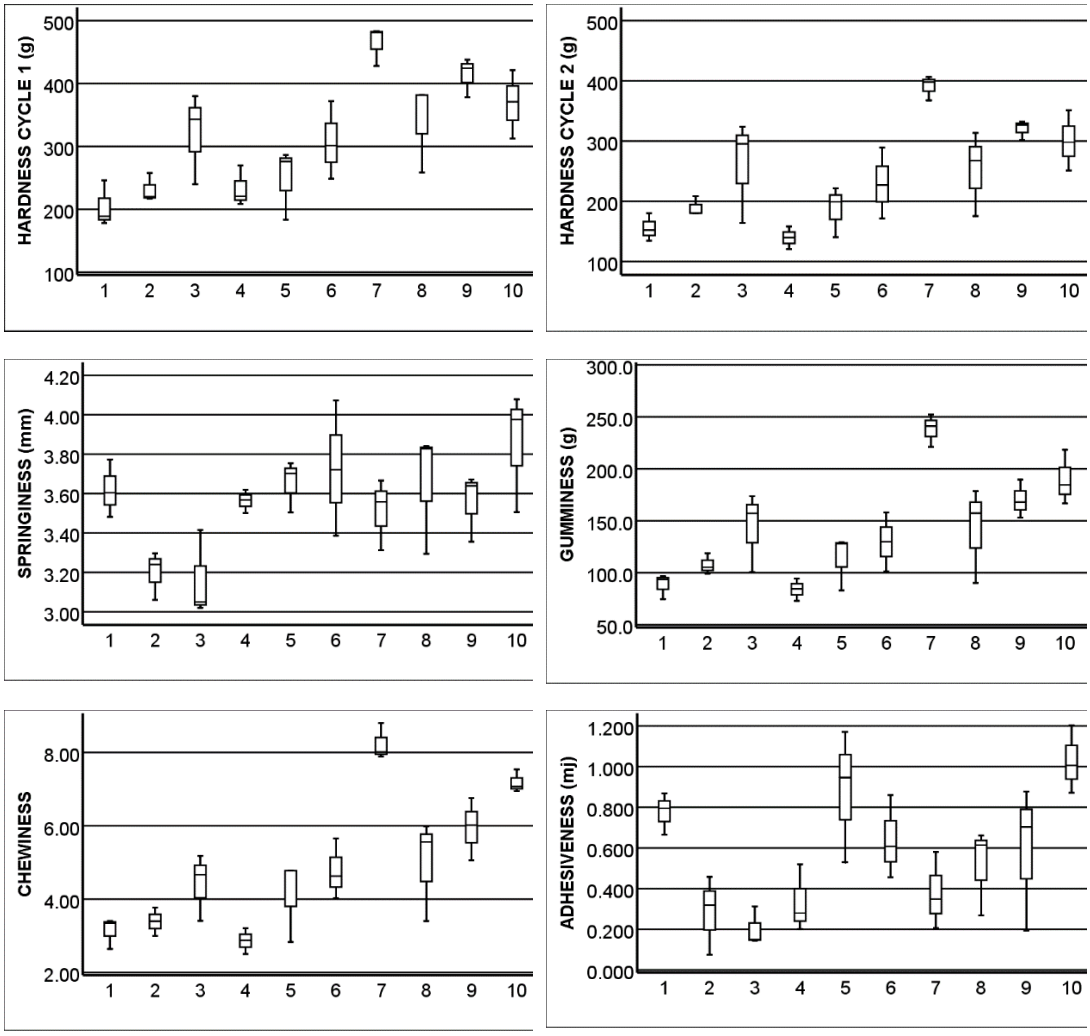

Figure 19: The variability of the texture parameters in the 10 studied date varieties (1) Barhi, (2) Boumann, (3) Dabbas, (4) Fardh, (5) Khalas, (6) Lulu red, (7) Neghal, (8) Reziz.

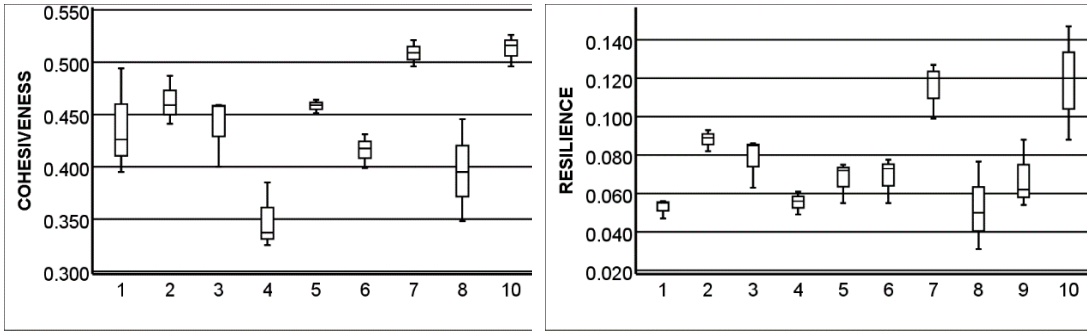

Figure 20: The variability of the texture parameters in the 10 studied date varieties (9) Sagei, and (10) Shishi (continued).

5.5 Discussion

The date fruits studied here were previously found to vary in their physico-chemical and textural properties [146]. In this study, it is shown that there is considerable cellular and tissue structural variation in date fruits (Figure 13). This structural variation in date fruits is mainly governed by the concentrations of the different fiber constituents and sugar types (sucrose versus reducing sugars). As the varieties studied here did not differ significantly in their contents of reducing sugars (Table 7), the main differences observed can be attributed to the fiber constituents. This is evidenced by the correlations between the fiber components and the textural properties of the fruits (Table 8).

The structure of the mature pericarp and the mode of its development in the date monospermous berry have been described previously [147-149]. A single-cell thin membrane, which constitutes a papery endocarp, is the innermost layer of the pericarp that surrounds the seeds [146]. The single-cell very thin tissue of the endocarp has no significance from a textural or nutritional point of view. The exocarp and mesocarp tissues of the date fruit are composed of different type of cells, including collenchyma, sclerenchyma, and parenchyma cells, as well as xylem vessels and phloem cells. Plant

cell walls are complicated supramolecular assemblies whose composition and molecular organization play major roles in cell protection, cell–cell adhesion, and intercellular exchanges [71, 150]. Cell walls are commonly composed of polysaccharides and lignin, but they also include some proteins and phenolic compounds, particularly ferulic acid [65, 151]. Primary cell walls are built around cellulose molecules that align to form microfibrils with crystalline and non-crystalline regions. The cellulose microfibrils are grouped into fibril aggregates, which a diameter of 10–25 nm, which are mounted by a matrix of hemicellulose and either pectin or lignin [152, 153]. Hemicelluloses, which are represented in date fruits by arabinoxylan and galactomannan (Table 7), are short-chain, amorphous polysaccharides that bind on the surface of cellulose microfbrils [154, 155] whereas pectin cross-links the hemicellulose molecules on adjacent microfbrils [156, 157]. Lignin is an amorphous complex phenolic polymer that is abundant in secondary cell walls where it contributes stiffness and strength [158]. Phenolic acids, particularly ferulic acid, form covalent bonds that cross-link lignin to the arabinoxylan [159, 160].

The fruit exocarp is primarily composed of epidermal, hypodermal, and sub-hypodermal layers (Figure 13.a-f). The hardness of date fruits is primarily due to the collenchyma and, especially, the sclerenchyma cells of the exocarp. Collenchyma cells are simple elongated cells that are extremely elastic, allowing the cells to expand with the growth of the fruit. The walls of collenchyma cells are largely hydrated cellulose, but small amounts of hemicellulose and pectin have also been reported [161, 162]. Collenchyma cells have unequally thickened primary cell walls, especially when observed in cross-sectional views [66]. The different thickness patterns of the walls are a characteristic feature, which is formed during elongation. It was reported that collenchyma cell walls contain many of the same polysaccharide components found

in parenchyma cell walls but the proportions and chemical species were distinctly different [163]. Sclereid cells are a reduced form of sclerenchyma cells, whose name is derived from scleros, which means hard, and they have highly thickened, lignified cellular walls. The sclerenchyma manifested as sclereid cells have very hard secondary cell walls that are thickened by lignin.

Table 8: Pearson correlation coefficients (r) for correlations between total fiber and fiber components and textural parameters of date fruits

Carbohydrate Polymer (%)	Hardness Cycle 1 (g)	Hardness cycle 2 (g)	Springiness (%)	Gumminess (g)	Chewiness (g)	Adhesiveness (mJ)	Cohesiveness	Resilience
Cellulose + β-glucan	NS	NS	NS	0.364*	NS	NS	NS	NS
Arabinoxylan	0.484**	0.558**	NS	0.624**	0.585**	NS	0.623**	0.531**
Galactomannan	0.433*	0.402*	NS	NS	NS	NS	NS	NS
Pectin	0.407*	0.411*	NS	0.477**	0.446*	NS	NS	NS
Klason lignin	0.651**	0.635**	NS	0.600**	0.523**	NS	NS	0.363*
Fructans	NS	NS	0.590**	NS	NS	0.611**	NS	NS
Total dietary fiber	0.704**	0.692**	NS	0.692**	0.635**	NS	NS	0.420*

The correlations are significant at ** p-value < 0.01, * p-value < 0.05, and NS indicates non-significant.

The parenchyma cells, the main cells of the mesocarp, have thin primary cell walls (Figure 16.i) that are composed of cellulose, arabinoxylan, galactomannan, and pectin. Depending on the variety and stage of maturation, the skin parenchyma may include aerenchymas, which are cells with large intercellular spaces, and chlorenchymas, which are cells containing chloroplasts, in immature fruits [164]. The storage parenchyma, with thin polyhedral primary cell walls, constitutes the dominant cells in the fruit mesocarp. These cells are usually the main components of the soft tissues, which function as reserves of starch during the early stages of fruit development and sugars upon maturity. Parenchyma cells can have intercellular spaces and be spatially arranged, or they can be compressed and tightly arranged. The inner white cells in the bottom of the mesocarp form inner fiber bundles, which are special parenchyma cells that are void of soluble sugars that function to provide strength without rigidity [67]. In a previous study, the inner fiber bundles of two date varieties, Fardh and Sagei, were analyzed and found that their cellulose+β-glucan, arabinoxylan, galactomannan, and pectin contents were comparable to those of the total fruit, but they lacked lignin [120]. The absence of a correlation between sugars and texture observed in these varieties is explained by the restricted range of variability in the type of sugars and their contents, making the effect of sugars on texture more of a constant than a variable [165]. Previously, the hardness of date fruit was found to correlate with pectin, crude fiber, and moisture contents, adhesiveness was found to correlate with glucose content, and gumminess was found to correlate with fructose, glucose, and total sugar content [143]. Thus, the mesocarp tissue is expected to contribute variably to fruit texture, depending on its composition and structural characteristics. For example, it is demonstrated that the xylem vessels in the mesocarp are lined with

helical coils, which are formed by the deposition of lignin and silica phytoliths in hollow vascular tracheids [166].

The instrumental TPA provides information about the mechanical properties of the fruit (Figure 18, Figure 20 and Table 8), which are related to the sensory properties perceived by humans [167, 168]. The values obtained for the texture properties in this study agree with previously reported texture property values for Saudi and Emirati varieties, including Barhi, Boumann, Khalas, Lulu, and Sagei, at the Tamr stage [36, 169]. Hardness or firmness, defined as the necessary force to attain a given deformation, is the most commonly assessed parameter of date fruit texture [143]. Lignin, which is the primary determinant of fruit hardness, is highly predominant in the skin layers of the fruits and particularly in the sclereids. Previously, the exocarp layer of Fardh and Sagei was found to contain two to four times the levels of total fiber and its different components in the whole fruit [120]. In this study, arabinoxylan, galactomannan, and pectin were found to correlate significantly with fruit hardness and the related parameters of gumminess and chewiness, but cellulose + β-glucan was only correlated with gumminess. Both lignin and arabinoxylan were correlated with resilience, and arabinoxylan exhibited a strong correlation with cohesiveness. Arabinoxylan has a water holding capacity that affects the cohesiveness and gumminess of bread dough [170]. Only fructan was found to be positively correlated with springiness. However, it also had a significant positive correlation with adhesiveness. Springiness logically follows cohesiveness. The addition of *Agave tequilana* fructans to oat cookies resulted in the formation of crystalline aggregates with lowered water adsorption and increased springiness and cohesiveness [171, 172]. Owing to their high sugar contents, date fruits may provide unique balance between adhesiveness and cohesiveness. Fruit hardness is influenced by the moisture content

of the fruit, but this parameter was not investigated here as it requires equilibration of the different varieties to known moisture levels, as shown before [173]. These authors differentiated two characteristics in dried date fruits: an “elastic nature,” which is characterized by deformation in the first compression, indicating hardness, adhesiveness, and chewiness, and a “plastic nature”, which is related to the ability of the fruit to regain its original shape after the first compression, indicating cohesiveness, resilience, and springiness. Because differences in moisture contents between these varieties were not large, the effect of moisture are excluded and the soluble sugars and fiber contents on a dry weight basis are considered. It has been found that adhesiveness/cohesiveness in sugary samples is a complex surface characteristic, which is related to stickiness and is not linearly related to moisture content [174]. Since Fracturability was not observed in the soft date varieties during compression, the measured cohesiveness may not be an “ideal cohesiveness” [173] which requires careful investigation in the future.

This is the first study demonstrating that the different fiber constituents correlate variably with different date fruit texture parameters (Table 8). The results will enable a better understanding of the sensory preference for different fruits [175] and they can be used in various food processing applications [176]. However, present study has at least two limitations that need to be addressed in future research. The first relates to the varieties used in the study, which, were of the inverted sugar type. Genetic studies have identified two populations of date varieties, an Eastern pool consisting of accessions from Asia to Djibouti (including UAE) and a Western pool consisting of accessions from Africa. The Eastern varieties produce ~77% soft dates and ~7% dry dates compared with the North African varieties, which produce ~52% soft dates and ~31% dry dates [177, 178]. As mentioned above, there is variability in the moisture

content of date fruits upon harvest [31, 32] and the soft varieties primarily store sugars as inverted sugars, glucose and fructose, whereas the dry varieties have variable levels of sucrose. It has been shown that there is a difference between crystalline sucrose and non-crystalline inverted sugars, especially fructose, in their ability to adsorb moisture with the presence of inverted sugars promoting a non-crystalline state leading to a plastic condition [179]. The second limitation is the disregard of the contribution of phenolic compounds to the texture of date fruits. Date fruits contain high concentrations and a wide range of phenolic compounds, including polymeric tannins that might contribute to date fruit texture [35, 142]. Inclusion of dry varieties in future research efforts will enrich the data and highlight the contribution of sucrose content/ratio as well as the textural attributes. On the other hand, the contribution of phenolic compounds seems to be small, and it will be complicated by the difficulty in their complete analysis and classification. Yet, it might add precision through small modifications of the fiber effects presented in this paper.

Chapter 6: Summary and Conclusion

6.1 Summary of research findings

In the present research work, the date fruit dietary fiber is studied extensively by quantitative and qualitative methods. The content of total and component dietary fiber in dates is quantified. The date fruit microstructure studies revealed the biomineralization of calcium and silicon in dates and the various morphological forms in which they are deposited in dates. The different lignification patterns in date fruits were also successfully identified. The research identified the date fruit component dietary fiber fractions that are important in contributing to the textural attributes of the fruit. The significant research outcomes, in short, are summarized below:

1) TDF content of date fruits is comparable to that of commonly consumed dry fruits.
2) Lignin is the main dietary fiber component in date fruits that accounts for about 1/3rd of the TDF. Lignin content is markedly high in harder date fruit varieties like Neghal than in soft date fruit varieties like Barhi.
3) The amount of pectin in soft date varieties like Barhi was lesser than those in hard date varieties like Neghal. This can be attributed to pectinolytic enzymes that act upon the middle lamella between cell walls. These enzymes degrade the pectin present and result in cell wall disintegration, making the fruits softer.
4) The biomineralized forms of calcium and silica were observed in date fruits. Calcium oxalate is deposited intracellular, as needle-like structures called raphides. Silica is deposited intracellularly in the xylem vessels and in the extracellular space as globular echinate phytoliths.

5) Lignification in date fruits in the xylem vessels and as secondary thickening of the sclereid cells or stone cells are observed. The lignification of xylem vessels as annular and helical coils was clearly observed by light microscopy and SEM.
6) The coexistence of silica and lignin in the xylem vessels of mature date fruits was suggested from the evidence obtained from microscopic studies of date fruit tissue.
7) The date fruit texture is correlated with some of the dietary fiber component fractions. Among the different dietary fiber constituents, lignin and arabinoxylan correlated with fruit hardness and the related textural parameters – gumminess, chewiness, and resilience. Arabinoxylan correlated strongly with cohesiveness, and pectin also contributes to fruit hardness.
8) The soluble sugars in dates were found not to have any correlation with the textural parameters.

A marked result of present research is the identification of a newly reported date fruit phytolith. Tracheary annulate/helical phytoliths in the shape of helical coils are observed in this study. Helical coils with a porous surface and articulated pitted tracheary phytoliths were observed for the first time.

6.2 Significance of the research

Much of the harvested dates and unharvested dates in UAE and around the globe are either being wasted or given away as cattle feed every year. Similarly, the date waste obtained from date processing factories after the removal of date syrup (dibs) can also be utilized to extract dietary fiber and dietary lignin. Harder date varieties can be utilized economically to recover dietary fiber, which can be utilized as a functional food ingredient in various food products. The physicochemical properties of the date

fiber can be utilized in food processing by effectively incorporating them as non-calorific bulking agents in foods to improve the food properties like viscosity in liquid foods and semi-solid foods, texture in solid foods, etc. The use of fiber additives can improve the sensory attributes and shelf-life of finished food products in general.

Knowledge of dietary fiber components in date fruits can result in the utilization of date fruit waste after extraction of date syrup (dibs) from the date processing industry. Date fruit waste after extraction can be effectively utilized in extracting functional ingredients. The date fruit waste after the production of dibs, one of the major processed products from dates in the country, can be utilized to extract dietary fiber, which can be used as an essential functional ingredient for the fortification of processed foods. The dietary fiber extract will be of low calorific value and is cholesterol-free. Such dietary fiber extracts can improve the physical and structural properties of foods besides making them healthy by reducing the total calories. These can be further purified and added to foods to improve their various nutritional and textural properties. E.g., date fruit waste from harder, dietary fiber-rich fruits can be effectively utilized to produce lignocellulosic extracts, which are very high in insoluble fiber fractions. This can be purified and used as a fiber additive in foods with or without bleaching to remove colors. Unbleached fiber extracts can be used in foods like brown bread, muffins, or cookies, and bleached fiber extracts in white bread, ice creams, etc. Similarly, fiber extracts from soft varieties can be utilized to produce soluble fiber extracts, which may be used in processed foods that require a soft textured product as the soluble fiber fractions can form a gel by imbibing water. Such extracts of pectin or arabinoxylan rich functional ingredient can be utilized to improve dough properties in bread dough, increase the total dietary fiber content of the final product, and improve

texture for products like baked goods, meat substitutes, frozen dairy, and other beverages.

Dietary lignin is identified as a functional food ingredient with radical scavenging and anti-cancerous properties [180]. Extracted lignin can be utilized for its antimicrobial properties and as a prebiotic ingredient in animal feeds [181]. Date fruits are identified as one of the richest sources of dietary lignin. The skin and the fruit cell wall are the major contributors of lignin fraction of the dietary fiber in dates. Date fruit waste and non-commercial date fruit varieties that are wasted because of their hard fruit texture at maturity can be effectively used for the extraction of purified lignin. Similarly, date fruits are the richest source of dietary silica. Silica, as discussed earlier, is found to have roles in the proper maintenance of bones, tendons, and blood vessels. Date fruit processing waste can also be utilized for the extraction of dietary fiber components rich in silica as it is predominantly found along with the deposited lignin fraction in the fruit tissue. Thus, this research works shed light on the utilization of date fruit waste from date processing industries and the underutilized date fruits of the country.

Biomineralization of date fruits with calcium and silica is proved to have roles in dealing with various biotic and abiotic stresses. This is very important in the desert conditions in which the trees are usually grown. Calcium and silica, along with lignin, might have significant roles in effective water utilization and coping with intense environmental stress. Regulating the genes responsible for biomineralization and lignification can result in varieties more resistant to harsh environmental conditions. The research outcome proving the contribution of various dietary fiber constituents to the fruit texture parameters will enable a better understanding of the sensory preference

for different fruit varieties among the consumers. This knowledge can be utilized in various food processing applications as well.

6.3 Recommendations for future research

The research facilitated exciting findings which can be further investigated in the future to make promising results in the field of food chemistry, nutritional, and health sciences. In the future forward, research can be progressed further at the following aspects:

1) Employing newer methods of DF analysis like AOAC 2011.25 to analyze the date fruit DF.
2) Development of productive, economical, and practical methods for the extraction of edible dietary fiber from dates. A method that is possible to upscale at the pilot level to the industry level.
3) Dates are the richest source of dietary lignin and dietary silica, and they are highly resilient. Hence further studies on the bioavailability and the plight of these components in human metabolism should be investigated in the future. Extraction and utilization of lignin and silica rich fraction from date fruit waste and wasted date fruits are to be investigated further for its effective utilization as functional ingredient in processed foods.
4) The mechanism of coexistence of lignification and silicification and its role in fruit texture might be studied in detail.
5) Use of a probe that is larger than the size of the sample taken in TPA analysis should be considered to effect proper compression of the sample.

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List of Publications

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